A BIOCHEMICAL STUDY OF RESISTANCE TO MILDEW IN OENOTHERA

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Science, in the University of Michigan.

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A BIOCHEMICAL STUDY OF RESISTANCE TO MILDEW IN OENOTHERA [1]

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[1] From the Laboratory of Plant Chemistry of the Department of Botany, University of Michigan, Ann Arbor, Michigan. This work was undertaken at the suggestion and under the direction of Prof. H. H. Bartlett, to whom I desire to express my indebtedness for his ever-ready help and invaluable advice during the progress of the work. I wish also to express my appreciation for assistance rendered by Dr. Frieda Cobb-Blanchard in the preparation of the record of the parentage of the plants that I examined, and to Mr. Peter J. Klaphaak for permitting me to use his *Oenothera* cultures and his field notes on them.

INTRODUCTION

THE NEED OF BIOCHEMICAL DATA IN THE SOLUTION OF THE PROBLEM OF IMMUNITY AND SUSCEPTIBILITY TO DISEASE IN PLANTS

The researches of recent years on the physiology of parasitism prove the ever-increasing need of biochemical data in the solution of the complex causes of immunity to disease in plants [see Appel(2) and Butler(8)]. Thus, Ward,(50) after having reviewed the literature dealing with the parasitism of fungi from the time that De Barƒy, Pringsheim, Koch, and Pasteur established the foundation of the parasite or germ theory of disease, reviews his own work on the Uredineae and makes the following statement regarding the conditions of parasitism in this group:

> Infection, and resistance to infection, depend on the power of the fungus protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins; and reciprocally, on that of the protoplasm of the cells of the host to form antibodies which destroy such enzymes or toxins, or to excrete chemotactic substances which repel or attract the fungus protoplasm.

Massee(31) holds the opinion that—

> the entrance of the germ tubes of a parasitic fungus into the tissues of a living, healthy plant depends on the presence of some substances, in the cells of the host, attractive to the fungus. In other words, infection is due to positive chemotaxis.

Wagner(49) indorses the view of Ward, saying that in some healthy plants three classes of antibacterial products are formed; namely, agglutinin, lysin, and substances limiting the multiplication of spores and bacteria.

Cook(12) reports that the loss of resistance to the attack of fungi shown by certain fruits after they have been removed from the trees is correlated with a decrease of enzymatic activity. Further, in collaboration with Wilson(14) he observes the toxic action of tannins and vegetable acids on the germinating spores of *Endothia parasitica* and related species.

Mains(30) indicates the close relationship of rust infection to the carbon metabolism of the host plant when he observes that, in the absence of light, the rust *Puccinia sorghi* is not able to develop in corn seedlings or pieces of corn leaf which have been deprived of their soluble carbohydrates. He, therefore, suggests that the obligate parasitism of the rusts is perhaps due to their requirement of particular organic compounds contained in the living host.

Gustafson(19) finds that the respiration of *Penicillium chrysogenum* increases up to a certain point with the increase of the hydrogen ion concentration of the medium and that the hydroxyl ion greatly inhibits respiration.

Hawkins and Harvey(21) observe that *Pythium debaryanum* Hesse secretes an enzyme that dissolves the middle lamella of the host tissues, and a toxin that kills the cells of the potato tuber; but nonchemical factors are also concerned with infection, for by a mechanical device these experimenters were able to prove that more pressure is required to puncture the tissues of the fairly resistant strains of the tuber than is required for the susceptible ones. More recently, Brown(7) reports that certain volatile substances given off from plant tissues have a distinct effect on the germination of the spores of *Botrytis cinerea*, some having a stimulating and others a retarding effect. He also obtained similar results by using ethyl acetate and various essential oils.

In another direction, where the specificity of plant pathogens in relation to their hosts is involved, we find Reed(40) pointing out the physiological specialization of parasitic fungi, and Thomas(48) presenting data to show that infection of *Apium graveolens* by *Septoria apii* is favored by conditions which accelerate the growth of the host. Raines,(39) in experimental work on certain rust diseases of the higher plants, concludes that the vegetative vigor of the host is a factor which influences its susceptibility and resistance to rusts. Allen,(1) as a result of her recent cytological studies of the infection of resistant and susceptible wheats by *Puccinia graminis tritici*, confirms the hypothesis that "immunity is due to definite antagonistic chemical interactions between host and parasite."

These various experimental findings are of extreme importance in the biochemical consideration of disease resistance. They prove two important points; first, the existence of particular chemical substances in plants which may be either toxic or stimulative to parasitic fungi and, second, the dependence of parasitic fungi upon specific biochemical conditions within their hosts. From a biochemical standpoint it is not hard to understand how certain highly specialized fungi are restricted to particular hosts, because only those hosts provide the special compounds used in their nutrition, or perhaps because only certain ones among a narrow range of possible hosts fail to secrete inhibiting substances when infection takes place.

Some very significant results have already been obtained in the biochemical study of immunity in plants, as may be seen from the extensive literature reviewed by Willaman and Sandstrom(51) coincidently with the publication of their own investigations on the biochemistry of plant diseases.

Jones(25) found cabbage to be resistant or nonresistant to the attacks of *Fusarium conglutinans;* Peltier(37) found wild relatives of the cultivated citrus fruits immune to citrus canker; Christensen(9) demonstrated the resistance of certain varieties of swede to *Plasmodiophora brassicae;* and Norton(34) developed strains of asparagus resistant to *Puccinia asparagi.*

Turning to the powdery mildews, Salmon (42, 43) found striking contrasts in the resistance of various species of *Hordeum* and of the genus *Bromus.* Klaphaak and Bartlett(27) found the same situation in strains and hybrids of *Oenothera* which are often the hosts of mildew strains referred to *Erysiphe polygoni* DC. It was the material of crosses prepared especially for the last-mentioned investigation that was made available to me.

THE OBJECT OF THE INVESTIGATION

This investigation concerns the problem of resistance to mildew in the genus *Oenothera.* The chemical compositions of several resistant and susceptible strains were compared, in order to discover whether or not there were definite correlation between composition and reaction to mildew. It was recognized, of course, that immunity might have a true biochemical basis, even if the gross analytical data failed to show differences. The power of a plant to secrete antibodies would not, for example, be indicated by such analytical work as that undertaken in the present investigation. If, on the contrary, there were large differences in the concentration of soluble carbohydrates or nitrogenous constituents, of such a nature as to have some obvious relationship to the nutrition of the fungus, the results would be of value. Again, differences in acidity, in the concentration of tannin, or of inorganic constituents, might be interpreted, if found, as a basis for corresponding differences in resistance. It was suspected, of course, when this investigation was undertaken, that such differences in gross chemical composition would be found. Whether they were or not, however, the analytical data would still be valuable for the light they would throw on the inheritance of chemical characteristics, on the fluctuating variation in chemical composition of the

same strain from year to year, and on the correlation between chemical composition and morphological characteristics of the several strains. The material was chosen so that the results would throw light on some of these matters, even if the chemical basis for disease resistance should not be disclosed.

THE ADAPTABILITY OF OENOTHERA FOR BIOCHEMICAL STUDIES OF DISEASE RESISTANCE

The choice of *Oenothera* for the investigation was determined not only by the sharp distinctions in disease resistance shown by the various strains, but also by the fact that there was available a great deal of genetically known material. Furthermore, the results would fit into a large body of genetical data regarding the same material and the usefulness of the results would thereby be enhanced. It was by no means necessary to use *Oenothera*, however, for strains differing with regard to disease resistance are found in most groups of plants.

It was possible to secure samples of resistant and susceptible *Oenothera* strains grown under identical conditions and collected at the same time, and thus to reduce to a minimum the possible effects of external factors. Klaphaak and Bartlett(27) noticed that—

> nothing is more characteristic of the various elementary species and hybrids than the great differences that they show in susceptibility to infection by mildew. * * * the differences shown by certain pairs of reciprocal hybrids, were astonishingly definite, the one being white with mildew and the other absolutely free, although both were grown in adjoining rows under identical conditions, and often with interlocking branches.

Moreover, in *Oenothera*, resistant strains or susceptible ones may be obtained at will, since resistance is inherited as a unit factor, and the rules governing the inheritance of the factor have been worked out for several strains in accordance with the hypothesis of heterogametism put forward by Bartlett(5) and by Cobb and Bartlett.(10) The experimental findings with regard to mildew resistance conform exactly to theoretical expectations and justify the zygotic formulae assigned to the strains by Klaphaak and Bartlett. It is not deemed necessary to explain here how immunity and susceptibility in *Oenothera* are formulated on the basis of this hypothesis, since such information has been published in the papers referred to. For the same reason I have omitted the descriptions of the morphological external characters of the elementary species and hybrids that I used for my experiments, as well as discussions of the

mildew which infects them, since such data are either on record already, or soon will be through the publications of co-workers.

It may be said, however, that in the strains which I have grown for my studies I noted that, in some cases of reciprocal hybrids, the mildewed one is vegetatively more vigorous than the resistant one. On the other hand, the leaves of the resistant hybrid are darker green and tougher than the susceptible one. It might seem offhand that the differences in susceptibility and immunity could be accounted for by some morphological characters of the forms, but Klaphaak and Bartlett,(27) after examination of sections of leaves from the five different species and hybrids chosen, stated that no such morphological differences of the leaves could be detected. Salmon,(42) in his studies of the barley mildew, *Erysiphe graminis* DC., presumably did not find any morphological differences of the host plant that would explain resistance, since he stated that "susceptibility and immunity were due to constitutional (physiological) peculiarities and not to any structural ones." De Istvanffi and Palinkas,(16) in their pathological studies of the vine mildew, *Plasmopara viticola*, express the opinion that—

> the susceptibility of the plant depends upon the supply of water in the organ, the vapour tension in the stomatic chambers and intercellular cavities generally, the turgescence of the cells, and to some extent on the chemical composition of the cell sap.

In view of these facts, the conclusion seems warranted that the resistance to infection by powdery mildew shown by a number of plants must have a physiological or biochemical explanation.

SOME PREVIOUS RESEARCHES BEARING ON THE PROBLEM

There has been no previous work, so far as I am aware, that deals with the biochemistry of resistance and susceptibility to any form of powdery mildew. Three papers which concern various aspects of the mildew problem are of interest in this connection. These are by Spinks,(46) Montemartini,(33) and Laurent.(28)

Spinks studied the effects of some inorganic salts on susceptibility of barley to mildew (*Erysiphe graminis*), and of wheat to yellow rust (*Puccinia glumarum*). He grew resistant and susceptible varieties of these two plants in Detmer's nutrient solution, with varying amounts of different salts. He concluded (*a*) that when the plants were provided with a large amount of nitrogen either in the form of ammonium salts or

nitrate, their susceptibility was increased, (*b*) that with lithium salts and mineral manures, especially the ones rich in potash, susceptibility was reduced, and (*c*) that plants which were semistarved with respect to nitrogen exhibited a considerable degree of immunity. Since Spinks's object was not strictly biochemical, he did not determine the amount of the different salts absorbed by the plants, or what became of the salts after they were absorbed. His findings, however, indicate the relationship of the nitrogen nutrition of the host plant to its susceptibility to the disease.

The relation of the nitrogenous constituents of the plant to mildew infection was also noted by Montemartini.(33) He observed that the water-soluble nitrogen in the leaves of the varieties of American oak resistant to powdery mildew constitutes only a little over 0.1 of the total nitrogen in the leaves. He said that this confirmed the previously published report of Pantanelli(35) that, in the susceptible oaks, the soluble nitrogen represents from 0.4 to 0.7 of the total nitrogen, while in resistant oaks it does not exceed 0.3.

Laurent(28) reported that severe infection of grapevines was noticed in the vineyards which had been manured directly with farmyard manure in the same year that the infection occurred, whereas in the ones in which phosphate and potassium fertilizers were used, infection was considerably reduced. Resistance was also found to vary according to the method of pruning and nipping, and the nature of the stock. He considered, therefore, that "all these factors act indirectly by modifying the chemical and physical qualities of the vine cells, resistance being due to the increase of the concentration of the vine-sap."

The strains of *Oenothera* used in the investigation are listed in Table 1.

MATERIAL

TABLE 1.—*List of elementary species and hybrids studied.*

Key No.	Culture No.	Name of strain.	Characteristic of strain.
1	0847	Resistant *Oe. pratincola* (a segregate from species hybrid)	Resistant.
1a	1827		
1b	2960		
2	0822	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible.
2a	1824		
2b	2959		
3	0864	*Oe. pratincola* hyb. *immunis*	Resistant.
3a	1826		
3b	2956		

TABLE 1.—*List of elementary species and hybrids studied*—Continued.

Key No.	Culture No.	Name of strain.	Characteristic of Strain.
4	0823	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible.
4a	1825		
4b	2954		
5	0830	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*	Resistant.
5a	1804		
6	0820	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*	Susceptible.
6a	1805		
7	0829	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*	Do.
8	1818	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant.
8a	2966		
9	1819	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible.
9a	2963		
10	0838	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*	Resistant.
11	0821	*Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago"*	Susceptible.
12	0814	*Oe. cinerescens* × *Oe. mississippiensis*	Resistant.
13	0827	*Oe. mississippiensis* × *Oe. cinerescens*	Susceptible.
13a	1800		
13b	2957		
14	1802	*Oe. cinerescens* × *Oe. mississippiensis* (metaclinic hybrid)	Do.
14a	2958		
15	0299	*Oe. pratincola* × *Oe. reynoldsii* (*Oe. pratincola* hyb. *amycosa*)	Resistant.
16	0177	*Oe. reynoldsii* × *Oe. pratincola*	Susceptible.
17	0842	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*	Do.
17a	1816		
17b	2962		
18	0849	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"*	Do.
18a	1817		
18b	2961		
19	0831	*Oe. mississippiensis* × *Oe. "biennis Chicago"*	Do.
19a	1806		
20	0841	*Oe. "biennis Chicago"* × *Oe. mississippiensis*	Do.
21	0839	*Oe. "biennis Chicago"* × *Oe. mississippiensis*	Do.
21a	1807		
22	0833	*Oe. mississippiensis* × *Oe. pratincola* hyb. *rubricalyx*	Do.
22a	1810		
23	0848	*Oe. pratincola* hyb. *rubricalyx* × *Oe. mississippiensis*	Do.
23a	1811		
24	1346	*Oe. pratincola* mut. *nitidissima*	Resistant.
24a	1348	*Oe. pratincola* mut. *nitidissima*	Do.
25	1347	*Oe. pratincola* mut. *nitidissima*	Susceptible.
25a	1349	*Oe. pratincola* mut. *nitidissima*	Do.
26	199	*Oe. numismatica*	Resistant.
27	1191	*Oe. pratincola* mut. *simulans*	Do.
28	1192	*Oe. pratincola* mut. *simulans rubricalyx*	Susceptible.
29	1829	*Oe. pratincola* hyb. *immunis*	Resistant.
29a	2953		
30	1231	*Oe. reynoldsii* × *Oe. pratincola*	Do.
31	167	*Oe. pratincola*	Susceptible.
32	1828	*Oe. mississippiensis*	Do.
33	1234	*Oe. reynoldsii*	Resistant.
34	2952	*Oe. pratincola* hyb. *rubricalyx*	Susceptible.
35	2950	*Oe. "biennis Chicago"*	Do.

COLLECTION OF MATERIAL

In all my experiments the leaves only were analyzed, because they are the parts of the plant which are most severely attacked by the mildew, and are mildewed earlier in the season than the stems. There seemed no object in carrying all the work through in parallel with stems, since the large amount of woody tissue would render variation in soluble constituents less easily detectable.

The first year's collection was made during the early part of September, 1920. Some of the leaves of the cultures had already shown signs of chlorophyll degradation, but the mildew could still be seen on the leaves of the susceptible strains. Since this was the time when the problem was planned, I decided to get the materials that were available and use them for finding out whether or not chemical differences between resistant and nonresistant leaves persisted. The data were, of course, only to be used as amplified by material of the following year, collected earlier in the season.

Individual plants of all strains selected during the first year were self-pollinated, and their progenies were grown in 1921. Leaves were again collected, but this time they were picked during the middle part of August, the period when the plants were observed to be in the developmental stage and when the mildew was most active. In this year I also collected leaves from some strains not represented in my first year's collection, for purposes that will be explained later on. In the third year, 1922, the cultures were confined to five pairs of reciprocal hybrids which were self-pollinated in 1921, and to three elementary species. The five pairs of reciprocal hybrids were the ones that had provided the best material during the first two years, while the three elementary species were genetically the same as the parents of the three pairs of reciprocal hybrids. The first year's analyses were made of hybrids already in the garden when the work was planned. The parents had been grown the year before. For complete data it was necessary to analyze material believed to be morphologically and genetically identical with the actual parents of the hybrids. Such strains had been carried on in the garden cultures, and provided material for analysis in the third year of the experiment after it had been conclusively shown that year after year the various strains maintain their chemical characteristics with the same fidelity that they maintain their morphological ones.

Two separate collections of leaves were made this time (1922), the first one from July 5 to July 19 when the susceptible strains were not yet infected by the mildew, and the second one from August 23 to August 31 when the susceptible strains were heavily infected.

The leaves were picked one by one from the main stems and branches of the plants and care was taken that a fairly representative portion of the full-grown leaves of each plant was collected, and that those plants in the cultures which appeared to be phenotypically different from the rest (twins or mutations) were left untouched. The number of individual plants in a culture representing one strain varied from one hundred to three hundred, and the leaves collected from all of them were mixed and rapidly dried in the sun. The dried leaves were placed in clean cardboard boxes and stored temporarily until they could be ground. They were milled to pass an 80-mesh sieve. The fine powder was then kept in dry glass jars. To make the material as comparable as possible, reciprocal hybrids were collected the same day, between 9 o'clock in the morning and 6 o'clock in the evening. It was not found necessary to brush the leaves for the purpose of removing any sand that might adhere to their surfaces for, since they were picked one by one, it was possible to avoid taking those which were not free from particles of foreign matter.

PLAN OF THE INVESTIGATION

The strains used can for convenience be considered in five groups (see Table 1). The first group contains the twelve strains and elementary species that were the parents of the reciprocal hybrids. The second group consists of seven pairs of reciprocal hybrids in which one hybrid of each reciprocal pair is resistant and the other one is susceptible. The third group is made up of three pairs in which both reciprocal hybrids are susceptible. The fourth consists of four strains of a single mutation, two of the four susceptible and the other two resistant. The fifth is composed of pairs of strains that have the same genetic composition but different pedigrees. These different groups of strains were used in order to answer the following questions: (*a*) Is there any difference in chemical composition between the resistant and susceptible strains of *Oenothera?* (*b*) If so, is there the same contrast in the chemical composition between reciprocal hybrids unlike in resistance to mildew that there is between wild elementary species of unlike resistance? (*c*)

In cases of mutations arising from the same parents and differing from one another with regard to resistance and susceptibility, are the visible differences between the forms paralleled in the chemical characteristics? (*d*) How does the chemical composition of their parents compare with that of hybrid progenies? (*e*) If two strains are judged by morphology and breeding behavior to have the same genetic composition, will they have also the same chemical composition?

Biochemical data bearing upon these five points were all desirable before any deduction could be made as to whether or not a chemical basis for resistance and susceptibility could be detected by the usual procedure of chemical analysis. It was planned to confine the investigation to the quantitative determination of the well-known chemical constituents of the plants, leaving for future consideration the study of specific constituents, if such there seemed to be. In order to reduce the analytical work, only four pairs of reciprocal hybrids were used in the study of the nitrogen distribution, the determination of carbohydrates, and the ash analysis.

Before proceeding to the description of the analytical methods adopted, I should explain why the study of the dried leaves was not supplemented by parallel investigations of the juices from the fresh plants. The juices were found to be so mucilaginous that the mechanical difficulties of obtaining sufficient material would have been almost insuperable in the case of an extensive investigation covering a large variety of strains. It seemed more logical to examine the tissues as a whole, leaving to the future more detailed researches, if the preliminary survey should indicate promising points of attack. More refined methods could be applied to more restricted material—perhaps a single contrasting pair of mutations or reciprocal hybrids, or a parent species and one of its mutations.

I am aware that during the process of drying there might have occurred autolytic changes which alter the chemical composition of the leaves. Since, however, the leaves were collected during summer days, they could be rapidly dried in one day so as to reduce autolysis to a minimum. Furthermore, since the experimental results were intended for comparative study any modification of the constituents of the leaves by autolysis would presumably be more or less of the same magnitude in all strains, because all the leaves were dried in the same way. Parkin(36) has studied autolytic changes in the composition of leaves during drying in connection with his studies of the car-

bohydrates of the foliage leaf of the snowdrop. He carried out two sets of experiments. Two equal portions of fresh leaves were taken. One portion was dried in the ordinary way, and the sugars from the dried leaves were determined. The other portion was immersed at once in liquid air, then withdrawn, and ground up while frozen and brittle. The powder was at once thrown into hot water to kill the enzymes, a little ammonia added to neutralize any acidity, and the sugars were determined in the aqueous extract. This procedure was designed to eliminate autolysis. The treatment with liquid air was merely to facilitate powdering of the leaves. It was the heat treatment, of course, which destroyed the enzymes. He found that the proportion of sugars in both cases is almost the same, as can be seen from Table 2.

TABLE 2.—*Parkin's data for different methods of sampling leaves for sugar analysis.*[a]

	Sucrose.	Hexoses (reducing sugars).	Total sugar.	Ratio of sucrose to hexose.
Experiment I:				
Fresh leaf	12.84	5.94	18.78	1:0.46
Dried leaf	12.74	5.67	18.41	1:0.44
Experiment II:				
Fresh leaf	10.46	12.87	23.33	1:1.23
Dried leaf	10.42	12.38	22.80	1:1.19

[a] The amounts of the sugars are calculated for 100 grams of dry leaf.

Any autolysis during drying would almost certainly have been reflected in the sugar ratios, since these afford an exceedingly sensitive indication of chemical changes going on in the cell. Parkin's work appears to justify fully the procedure adopted in sampling.

METHODS OF ANALYSIS

Moisture.—Duplicate samples of the powdered leaves, the weight varying from 1.5 to 2.0 grams, were heated in a vacuum oven at from 90° to 95° C. for six hours. The loss of weight was taken as the moisture content.

THE NITROGEN PARTITION

Total nitrogen.—It was decided to use a combination of the Gunning method modified to include the nitrogen of nitrates(26) and the iodometric method of Willard and Cake(53) for the determination of amino nitrogen in organic substances. The

nitrate nitrogen of the sample was retained by using salicylic acid and the nitro compound resulting from this treatment was reduced with sodium thiosulphate. The digestion was then carried out in the usual way with concentrated sulphuric acid and potassium sulphate, except that after the addition of potassium sulphate boiling was continued only for an hour at most. The digestion was completed by the addition of potassium persulphate free from ammonium salts, the amount varying from five to ten times the weight of the sample taken. When the destruction of the non-nitrogenous organic matter was complete, the acid solution was made alkaline with sodium hydroxide and the ammonia determined by oxidation to nitrogen with standard sodium hypobromite solution. This was done by adding an excess of the hypobromite to the alkaline solution and titrating the excess back with 0.2 *N* sodium thiosulphate after the addition of potassium iodide and hydrochloric acid.

Ammonia nitrogen.—The determination was made according to Grafe's method.(18) Ten grams of the sample were transferred to a long-necked 500-cubic-centimeter Kjeldahl flask, together with 20 cubic centimeters of concentrated sodium chloride solution, 10 cubic centimeters of 95 per cent ethyl alcohol, 100 cubic centimeters of distilled water, 10 cubic centimeters of saturated sodium carbonate solution (or enough to render the mixture alkaline), and 0.3 cubic centimeter of caprylic alcohol. The mixture was subjected to distillation in vacuo at a temperature ranging from 35 to 37° C. for three hours. The distillate was caught in a known volume of 0.1 *N* sulphuric acid, and the excess acid was titrated back with 0.1 *N* sodium hydroxide, using methyl orange test solution as an indicator. Caprylic alcohol was used to prevent excessive foaming, since it was found that the amount of ethyl alcohol indicated by Grafe was not sufficient for the purpose. A blank determination was carried out and the result subtracted from that given by the sample.

Protein nitrogen.—The protein nitrogen was determined according to Stutzer's method as adopted by the Association of Official Agricultural Chemists for albuminoid nitrogen.(26)

Free amino acid nitrogen.—Two or three grams of the powder were weighed, placed in a 300-cubic-centimeter Erlenmeyer flask, 100 cubic centimeters of distilled water added, and the flask shaken in the shaking machine for half an hour. The content of the flask was transferred to a Buchner funnel and filtered. Of the aqueous extract, two aliquot portions of 10 cubic centi-

meters were pipetted and used for the determination according to Van Slyke's method.(56)

The rest of the nitrogen, namely, the nitrate, acid amide, humin, basic, nonbasic, and peptide, was determined in the aqueous extract which was prepared from the leaves in the following way:

A 15-gram sample of the powdered leaves was placed in a liter beaker and boiled for thirty minutes with 500 cubic centimeters of distilled water. When cold, the clear supernatant liquid was carefully decanted from the sediment. The treatment was repeated twice, with 250 cubic centimeters of water each time. The boiling was confined to fifteen minutes. The three aqueous extracts were combined and rendered acid with a few drops of acetic acid and then boiled for a few minutes to coagulate the soluble proteins. While hot, the liquid was filtered under pressure through an ordinary filter paper supported underneath by linen. A hardened filter paper could not be used because of the mucilaginous nature of the aqueous extract. When the extract was cold, it was made up to a volume of 1,000 cubic centimeters. No preservative was used in the extract, as the experiment was so arranged that all the determinations were started the same day. The object of setting aside the powdered leaves after they were boiled with water was to allow the fine particles of the powder to settle out so that the clear mucilaginous aqueous extract could be removed by decantation. The method generally followed of filtering the aqueous extract of the leaves immediately after boiling was also tried, but was given up because the extract was so viscous that filtration was very slow.

Water-soluble nitrogen.—Two 50-cubic-centimeter portions of the aqueous extract were transferred to Kjeldahl digestion flasks and evaporated almost to dryness. The total nitrogen was determined in exactly the same way as in the leaf powder, except that 15 cubic centimeters of sulphuric acid, 1 gram of salicylic acid, and 2.5 grams of sodium thiosulphate were used.

Nitrate nitrogen.—The determination was carried out according to Scales's method as modified by Harrison.(20) The principle is the reduction of the nitrate nitrogen to ammonia in alkaline solution by means of some reducing agent and, after reduction, distillation into a known amount of acid, the excess of which is found by titration. Harrison(20) has pointed out the advantage of Scales's method over that of Devarda. Since the method was to be used for the nitrate nitrogen in plant ex-

tracts, it was necessary to determine first whether other forms of nitrogen in the extract might also be included. A series of experiments was conducted, using an extract of the sugar beet which was available at that time. Nitrogen in the form of acid amide, amino acid, nitrate, nitrite, and ammonia was added to known volumes of the extract and the amount of nitrogen recovered from each of them was determined. The results of the experiments are shown in Table 3.

TABLE 3.—*Results of nitrate nitrogen determination.*

	N/14 H_2SO_4 consumed.	Nitrogen recovered.	Nitrogen present.	Nitrogen recovered.
	cc.	*mg.*	*mg.*	*Per cent.*
50 cc. of extract	48.90			
50 cc. extract + 0.625 gm. sugar	48.87			
50 cc. extract + 0.25 gm. asparagine + 0.1992 gm. acetanilide	50.90	2.00	73.60	2.71
50 cc. extract + 0.25 gm. asparagine + 0.625 gm. sugar	50.30	1.40	53.00	2.64
50 cc. of extract + 0.25 gm. asparagine	50.70	1.80	53.00	3.39
50 cc. extract + 0.625 gm. sugar + 0.40 gm. alanine	48 70	0.00	62.90	0.00
50 cc. extract + 0.075 gm. KNO_3	59.10	10.20	10.38	98.26
50 cc. extract + 0.0375 gm. KNO_3	54.20	5.30	5.19	102.11
200 cc. distilled water + 0.2038 gm. KNO_3	28.30	28.30	28.20	100.35
200 cc. distilled water + 0.3247 gm. $NaNO_2$	64.30	64.30	65.91	97.55
200 cc. distilled water + 0.1179 gm. NH_4NO_3	39.30	39.30	40.12	97.95
50 cc. extract + 0.05895 gm. NH_4NO_3	68.62	19.72	20.06	98.60

The results indicate that only a small percentage of the nitrogen from acid amides, as in acetanilide, is determined as nitrate by this method. The amino acid nitrogen, as in alanine, remains unaffected. The same holds true, of course, for the two types of nitrogen in asparagine. As was expected, the method accounts for all the nitrogen from nitrites and ammonia—a fact which must be taken into account in using the method. In *Oenothera* leaves, no nitrite was found, so the nitrate nitrogen as determined by this method includes only the ammonia nitrogen. Since the latter is also known, the nitrate nitrogen can be found by difference. The method as adopted was therefore as follows: Two 200-cubic-centimeter portions of the aqueous extract were transferred to 500-cubic-centimeter Kjeldahl digestion flasks which contained 80 grams of zinc-copper couple. Then 6.5 grams of sodium chloride magnesium oxide mixture (5 grams of sodium chloride to 1.5 grams of magnesium oxide) were added to the contents of the flasks and the distillation carried

out. The distillate was collected in a known amount of 0.1 *N* sulphuric acid, until 150 cubic centimeters of the distillate were obtained. The excess of acid was determined by titration. A blank experiment, using 200 cubic centimeters of distilled water, was also carried out.

The acid amide, humin, and basic and nonbasic nitrogen of the aqueous extracts were determined according to the method of Hausmann as adopted by Jodidi.(22)

Peptide nitrogen.—A 50-cubic-centimeter sample of the aqueous extract was treated with 10 cubic centimeters of concentrated hydrochloric acid and kept boiling under a reflux condenser for three hours. The acidified extract was evaporated to dryness on the water bath to drive off the acid, and the residue was treated with a small amount of water and filtered. The filtrate was evaporated to about 10 cubic centimeters on the water bath and the nitrogen of the amino acids determined according to Van Slyke's method.(56) The nitrogen found comes from the free amino acids present in the leaves and from the amino acids formed by the breaking down of the peptide linkings by hydrolysis. To obtain the peptide nitrogen, the amino acid nitrogen found before hydrolysis was subtracted from that found after hydrolysis.

The nitrogen partition in the leaves after acid hydrolysis, was carried out according to Jodidi and Moulton's procedure(23) as applied to spinach.

THE CARBOHYDRATES

Sugar.—In the determination of the sugar content of the leaves, effort was first directed to finding an appropriate process of extraction. The cold-water method was first tried but was abandoned because, owing to the slimy nature of the aqueous extract, the leaves could not be extracted repeatedly with cold water; in the second extraction, the extract would hardly pass through the filter. The same difficulty was met when hot water was tried. The use of a dilute solution of alcohol was therefore resorted to. This was found to be a satisfactory solvent. Accordingly, a series of experiments was carried out to determine the strength of alcohol to be used and the length of time required for extraction. Three different strengths of alcohol were tried, and the samples were extracted three successive times. The period of shaking for each extraction was two hours for the first and second series and one hour forty-five minutes for the third and fourth. In the fourth series, in which 10 per cent alcohol was used, it was found that the concentration was not high

enough to prevent the extract from becoming slimy. In all cases the alcoholic extract was made up to a definite volume, and the reducing sugar in an aliquot portion of the clarified solution was determined before and after inversion. The reducing sugar was determined by means of Fehling's solution, the precipitation being carried out according to the directions of Munson and Walker,(26) and the copper in the precipitate estimated according to the iodometric method of Low.(26) The results obtained are given in Table 4.

TABLE 4.—*Results of sugar extraction under various conditions.*

Sample No.	Time of shaking.		Alcohol.	Reducing sugar before inversion.	Reducing sugar after inversion.	Reducing sugar freed by hydrolysis.
	Hrs.	*min.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0842—1	6		25	7.42	10.36	2.94
0842—2	6		25	7.46	10.38	2.92
0842—3	6		15	7.41	10.30	2.89
0842—4	6		15	7.44	10.32	2.88
0842—5	1	45	15	7.48	10.38	2.90
0842—6	1	45	15	7.44	10.37	2.93
0842—7	1	45	15	7.34	10.36	3.02
0842—mean				7.43	10.35	2.92

Since the reducing sugar obtained in all series was almost the same, there was no advantage in using a 25 per cent alcohol, nor in allowing the extraction to proceed for a long period of time. To insure perfect extraction of the sugar, it is of course preferable to use a low concentration of alcohol. Since a 10 per cent strength removed some mucilage and was therefore unsatisfactory, the 15 per cent alcohol was chosen for further work.

Since in the previous experiments no precautions were taken to destroy or to inhibit the action of the enzymes present in the leaves, the results were compared with those obtained by using a very strong solution of alcohol as a solvent. A known weight of the sample was placed in a Soxhlet extractor and extracted first with anhydrous ether to remove the chlorophyll and other, fatty substances. The powder was dried and then extracted with 95 per cent alcohol for forty-eight hours. The alcohol of the extract was recovered by distillation under reduced pressure, and the residue was dissolved in water and made up to a definite volume. The reducing sugar in the clarified solution of an aliquot portion of the extract was determined in the same way as in the previous experiments. The residual leaves were dried and extracted with water, to remove the sugar which was not

dissolved by the alcohol, and the reducing sugar in the aqueous extract was determined. The results obtained are shown in Table 5.

TABLE 5.—*Comparison of analytical results for sugar with and without taking precautions for the inactivation of enzymes.*

	Sample No.	Reducing sugar before inversion.	Reducing sugar after inversion.	Reducing sugar freed by hydrolysis.
		Per cent.	*Per cent.*	*Per cent.*
Ninety-five per cent alcoholic extract	0842–A	5.66	7.84	2.18
Aqueous extract	0842–A	1.91	2.66	0.75
Total sugar; enzymes inactivated	0842–A	7.57	10.50	2.93
Ninety-five per cent alcoholic extract	0842–B	6.48	8.97	2.49
Aqueous extract	0842–B	1.08	1.44	0.36
Total sugar; enzymes inactivated	0842–B	7.56	10.41	2.85
Mean results of total sugar; enzymes inactivated	0842	7.57	10.46	2.89
Mean results from Table 4; enzymes not inactivated	0842	7.43	10.36	2.92

The sugars found by using strong hot alcohol, to prevent the action of the enzymes, were practically in the same proportions as when weak alcoholic solutions were used. Only a very small portion of the sugars in *Oenothera* leaves is present as non-reducing sugar; hence the enzymatic effect was not appreciable. Furthermore, the presence of even a low concentration of alcohol might possibly retard the enzymatic action.

Since the ordinary method of extraction with a weak solution of alcohol is rapid and yields the same results as more complicated procedures, the method adopted for extraction of the sugars was as follows: A 5-gram sample was transferred to an Erlenmeyer flask, 250 cubic centimeters of 15 per cent alcohol added, and the flask shaken in a shaking machine for one hour. The extract was filtered by suction. The powder with the filter paper was returned to the original flask, 150 cubic centimeters of the alcoholic solution were added, and the flask was shaken for thirty minutes. The extract was filtered and the process repeated, using 100 cubic centimeters of the alcoholic solution and shaking for fifteen minutes. The combined alcoholic extracts were made up to a volume of 500 cubic centimeters. An aliquot portion of the extract was at once withdrawn, clarified with lead subacetate solution, and the excess lead removed with sodium carbonate. The reducing sugar in the clarified solution before and after inversion was then determined according to the method already described.

Pentosan.—The pentosan was determined in a sample of the dried leaves according to the method adopted by the Association of Official Agricultural Chemists for pentosans in foods and feeding stuffs.(26)

Starch.—In *Oenothera* leaves tannins occur in such considerable amounts that they must be removed before the starch can be determined by the diastase method with subsequent acid hydrolysis. Accordingly, a given amount of the powdered leaves was extracted in a Soxhlet extractor with 95 per cent alcohol for forty-eight hours. The powder was allowed to dry and then treated with cold water to remove any residual tannin that is insoluble in 95 per cent alcohol. The powder after undergoing this treatment was transferred to a beaker, 50 cubic centimeters of water added, and the starch determined according to the directions given in the Journal of the Association of Official Agricultural Chemists,(26) except that the dextrose resulting from the hydrolysis of starch was estimated according to the method adopted for the free reducing sugar.

Tannin.—The tannin was determined according to the Proctor modification of the Löwenthal method as applied to tea by the Association of Official Agricultural Chemists.(26) In *Oenothera* leaves, which give a mucilaginous aqueous extract, it was found best to set aside the leaf infusion overnight, after which the supernatant liquid could be decanted.

Crude fiber.—A 2-gram sample of the leaves was extracted with ordinary ether in a Soxhlet extractor until the percolate was colorless. The leaves were dried and the crude fiber determined according to the directions given in the Journal of the Association of Official Agricultural Chemists for crude fiber in foods and feeding stuffs.(26)

The water-soluble acids.—A 5-gram sample of the leaves was transferred to an Erlenmeyer flask, 200 cubic centimeters of distilled water added, and the whole shaken for thirty minutes. The extract was filtered by suction. A 20-cubic-centimeter aliquot portion of the filtrate was diluted with 100 cubic centimeters of distilled water and titrated with 0.1 *N* sodium hydroxide using phenolphthalein as an indicator. The total acids found were expressed in terms of the number of milligrams of sodium hydroxide required to neutralize the acids from one gram of moisture-free sample.

Total ash.—A sample, weighing 2 to 3 grams, was incinerated in a platinum crucible at a low heat until it was reduced to a gray ash. It was cooled, treated with hot water to dissolve the

soluble salts, and filtered. The filter paper with the carbonaceous residue was transferred to the original crucible, dried, and heated to full redness until the ash was white. The filtrate was evaporated to a small volume on the water bath and the concentrated solution was added a little at a time to the crucible containing the insoluble ash and the whole evaporated to dryness on the water bath. A few cubic centimeters of strong ammonium carbonate were added in order to change the alkaline earth oxides into their carbonates. The crucible was then heated in a free flame to a very dull red heat until a constant weight was obtained. The ash is reported as containing carbonates.

THE INORGANIC CONSTITUENTS OF THE ASH

A 30-gram sample of the leaves was incinerated at a low heat in a porcelain dish until a gray ash was obtained. The ash was then treated in the same way as in the determination of the total ash. In this case, of course, a large platinum dish was used. The ash obtained was pulverized and kept in a well-stoppered bottle. An aliquot portion of the ash, weighing from 2.5 to 3 grams, was treated with 200 cubic centimeters of 10 per cent hydrochloric acid and the solution evaporated to complete dryness on the water bath in order to dehydrate the silica. The residue was taken up with 100 cubic centimeters of 10 per cent hydrochloric acid and 200 cubic centimeters of warm water. It was filtered, washed thoroughly with water, and the filtrate after cooling was made up to 500 cubic centimeters. In the subsequent determinations, this filtrate was referred to as the original solution.

Silica.—The residue obtained in the preparation of the solution of the ash was treated according to directions given in the official methods of the Association of Official Agricultural Chemists for silica in plants.(26) The silica was estimated by the loss of weight of the residue when treated with hydrofluoric acid.

Calcium.—A 100-cubic-centimeter sample of the original solution was taken and the calcium precipitated as oxalate according to directions given by Mitchell.(32) The calcium oxalate was dissolved in hot 10 per cent sulphuric acid and at once titrated with 0.1 *N* potassium permanganate.

Magnesium.—To the filtrate and washings from the calcium determination, 25 cubic centimeters of concentrated nitric acid were added and the acid liquid was evaporated to complete dryness on the water bath. The residue was taken up with dilute

hydrochloric acid and the magnesium was precipitated as magnesium ammonium phosphate according to the method given by Mitchell.(32) The precipitate was ignited, and weighed as magnesium pyrophosphate.

Phosphoric acid.—The phosphoric acid was determined in 50 cubic centimeters of the original solution by one of the methods given in the Journal of the Association of Official Agricultural Chemists,(26) in which the phosphoric acid was precipitated as ammonium phosphomolybdate, and the precipitate was dissolved in a known excess of 0.1 *N* sodium hydroxide, the excess alkali being determined by titration.

Sulphuric acid.—The determination was carried out according to directions given in the official methods of the Association of Official Agricultural Chemists, 100 cubic centimeters of the original solution being used.

Potassium and sodium.—The filtrate and washings from the sulphuric acid determination were treated according to the directions given in the official methods of the Association of Official Agricultural Chemists(26) for potassium and sodium in plant products. When the potassium and sodium chlorides were obtained, the separation of potassium was accomplished by the perchlorate method, following in detail the directions given by Willard.(52)

PRESENTATION OF RESULTS

In all the tables the results are the mean of two determinations which agreed with each other within the limit of experimental error. Unless otherwise stated, the results are expressed as percentages of the moisture-free samples. In the list of the strains studied and in the tabulation of results, the pistillate parent is named first and then the pollen parent in all strains which resulted from crosses.

THE NITROGEN DISTRIBUTION

The chief nitrogenous compounds from the point of view of their importance in the life processes of the plant are the proteins, amino acids, peptides, acid amides, nitrates, and some of the nitrogen bases. The nitrogen source for the parasitic fungi is of course the body of the host. The fungi may conceivably obtain their nitrogen either by direct absorption of the soluble simpler nitrogenous compounds or by the digestion of colloidal compounds, which are, as far as known, proteins.

The major portion of the nitrogenous constituents of the plant is protein which, at least as far as storage protein is concerned, is continually being decomposed into amino-acid units and again resynthesized. In a diseased plant, the fungus must disturb the normal nitrogen distribution, even if it uses only amino acids produced in a normal manner by the host, whether by primary synthesis or by autodigestion of protein. Disturbance might be even greater if the fungus excreted proteases which digested the plant proteins independently of the normal metabolism of the host. It is, therefore, a logical expectation that the nitrogen distribution in healthy plants will not be the same as it is in diseased plants.

Among the first investigators to find significant differences in the nitrogen distribution between healthy and diseased plants was Jodidi.(22) He studied the nitrogenous constituents of healthy and mosaic cabbage, and with Moulton and Markley(24) extended his investigation to the mosaic disease of spinach. In both plants the diseased individuals were found to have a lower percentage of total nitrogen, acid amide, monoamino and diamino-nitrogen than the normal ones. Moreover, nitrous acid was present only in the diseased plants. These characteristic features of the diseased cabbage and spinach were ascribed to denitrification in the affected tissues, by which nitrates were reduced in part to ammonia and in part to nitrites. The latter reacting with the amino groups of the various organic compounds brought about the elimination of elementary nitrogen. There is room for doubt as to the correctness of Jodidi's explanation. However, his results show conclusively that in mosaic plants the nitrogen distribution is different from that in normal ones. Since no causal organism has been demonstrated for the mosaic diseases, there was no reason to look for findings parallel to Jodidi's in the present investigation of mildewed *Oenothera*. The results are, in fact, very diverse. As far as I know, no previous investigation has dealt in a thoroughgoing way with a known fungus infection from the standpoint of nitrogen distribution.

Total nitrogen.—In *Oenothera*, there exists a remarkable difference in the proportion of the various groups of nitrogen compounds in the resistant and susceptible strains. First of all, let us consider the results obtained for total nitrogen, which are shown in Table 6.

TABLE 6.—*Percentage of total nitrogen in the leaves of different strains of Oenothera.*

Key No.	Name of strain.	Characteristic of strain.	Nitrogen in 1920.	Nitrogen in 1921.	Nitrogen in 1922.
			Per cent.	*Per cent.*	*Per cent.*
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	2.55		
1a				2.59	
1b					2.54
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	3.39		
2a				3.61	
2b					3.03
3	*Oe. pratincola* hyb. *immunis*	Resistant	2.59		
3a				2.79	
3b					2.29
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	3.55		
4a				3.87	
4b					3.08
5	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*	Resistant	3.03		
5a				3.25	
6	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*	Susceptible	3.38		
6a				3.57	
7	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*	Resistant	3.00		
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	do		3.22	
8a					2.45
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible		3.65	
9a					2.81
10	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*	Resistant	3.13		
11	*Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago"*	Susceptible	3.62		
12	*Oe. cinerescens* × *Oe. mississippiensis*	Resistant	2.64		
13	*Oe. mississippiensis* × *Oe. cinerescens*	Susceptible	2.93		
13a				2.93	
13b					2.69
14	*Oe. cinerescens* × *Oe. mississippiensis* (metaclinic hybrid)	do		2.86	
14a					2.64
15	*Oe. pratincola* × *Oe. reynoldsii* (*Oe. pratincola* hyb. *amycosa*).	Resistant	2.22		
16	*Oe. reynoldsii* × *Oe. pratincola*	Susceptible	2.71		
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*	do	3.13		
17a				2.93	
17b					2.68
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"*	do	3.74		
18a				3.16	
18b					2.81
19	*Oe. mississippiensis* × *Oe. "biennis Chicago"*	do	2.88		
20	*Oe. "biennis Chicago"* × *Oe. mississippiensis*	do	3.25		
21	*Oe. "biennis Chicago"* × *Oe. mississippiensis*	do	3.08		

TABLE 6.—*Percentage of total nitrogen in the leaves of different strains of Oenothera*—Continued.

Key No.	Name of strain.	Characteristic of strain.	Nitrogen in 1920.	Nitrogen in 1921.	Nitrogen in 1922.
			Per cent.	*Per cent.*	*Per cent.*
24	*Oe. pratincola* mut. *nitidissima*	Resistant		2.79	
24a	do	do		2.92	
25	do	Susceptible		3.23	
25a	do	do		3.16	
26	*Oe. numismatica*	Resistant		2.40	
27	*Oe. pratincola* mut. *simulans*	do		2.34	
29a	*Oe. pratincola* hyb. *immunis*	do			2.54
33	*Oe. reynoldsii*	do		2.28	
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible			2.95
35	*Oe.* "*biennis Chicago*"	do			2.91

(*a*) For all pairs of reciprocal hybrids, the susceptible members of the pairs contain a higher percentage of total nitrogen than do the resistant ones. This difference persisted in the strains grown for three consecutive years.

(*b*) The same statement holds true for elementary species. The resistant species are found to have less total nitrogen than the susceptible species.

(*c*) In *Oenothera pratincola* mut. *nitidissima* (key Nos. 24–25), which occurs in susceptible and resistant forms, the susceptible form has a higher total nitrogen than the resistant one. This mutation, as has been stated elsewhere in this paper, has been recovered in many progenies of crosses into which it entered, unchanged except for modification of its susceptibility.

(*d*) Pairs of strains which have the same genetic composition but belong to different pedigrees, contain almost the same percentage of total nitrogen. This fact is shown in the crosses *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis* (key Nos. 5 and 7) and *Oe. mississippiensis* × *Oe. "biennis Chicago"* (key nos. 19 and 21).

(*e*) A striking situation is provided by the reciprocal hybrids from crosses between *Oe. cinerescens* (immune) and *Oe. mississippiensis* (susceptible). The hybrid (key No. 12), in which *Oe. cinerescens* is the pistillate parent, is resistant and therefore has a lower total nitrogen than the reciprocal hybrid *Oe. mississippiensis* × *Oe. cinerescens* which is susceptible (key No. 13). But the metaclinic hybrid *Oe. cinerescens* × *Oe. mississippiensis* (key No. 14), being of the type *Oe. mississippiensis*, is susceptible. We should expect it to show a higher total nitrogen than the resistant matroclinic plants (key No. 12) and this is exactly the case, for the percentage of total nitrogen found is almost the

same as in its reciprocal hybrid, where *Oe. mississippiensis* is the pistillate parent (key No. 13). The two were grown for two successive years, and the difference in their total nitrogen is very slight. The data for the F_2 generation of the resistant hybrid are lacking, as the self-pollinated matroclinic plant of the F_1 gave no seeds. Genetical data regarding this metaclinic hybrid are given by Klaphaak and Bartlett.(27)

(*f*) In pairs of reciprocal hybrids in which both members are susceptible, the total nitrogen is comparatively high and the difference between reciprocals is slight. The only exception is found in the results for 1920 of the crosses between *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*, where the variation in total nitrogen is rather high. But the same reciprocal hybrids, when grown in 1921 and 1922, show much less difference. Since the collection for 1920 was made when the plants were approaching senescence, presumably the cause of the variation is traceable to this fact. Hybrids of this type are shown in Table 6 under key Nos. 17 and 18 and Nos. 19 and 20.

(*g*) In crosses between resistant and susceptible parents, the resistant hybrid and the resistant parent exhibit a low percentage of total nitrogen. The reverse is true for the susceptible members, which both have high total nitrogen. Thus, resistant *Oe. pratincola* (key No. 1), a segregate from a species hybrid, and its reciprocal hybrid, mildewed *Oe. pratincola* (key No. 2), come from *Oe. pratincola* hyb. *immunis* (key No. 29a) and *Oe. pratincola* hyb. *rubricalyx* (key No. 34). Resistant *Oe. pratincola*, according to Klaphaak and Bartlett,(27) has the zygotic composition $\alpha\hat{\imath}\beta$I and hence it is immune. (The formation takes account only of the immunity factor.) Its resistant parent, *Oe. pratincola* hyb. *immunis*, has also the zygotic composition $\alpha\hat{\imath}\beta$I. Both of them have 2.54 per cent total nitrogen. The mildewed *Oe. pratincola* has also the same zygotic composition as its susceptible parent, *Oe. pratincola* hyb. *rubricalyx*, which is $\alpha\hat{\imath}\beta\iota$, and their total nitrogen varies only very slightly. The hybrid has 3.03 per cent and the parent has 2.95 per cent.

(*h*) In crosses between two susceptible parents, the resultant hybrids, which are both susceptible, agree rather closely with their parents in having a proportionately high total nitrogen. This fact is illustrated by crosses between *Oe. pratincola* hyb. *rubricalyx* and *Oe. "biennis Chicago"* (key Nos. 17b, 18b, 34, and 35).

TABLE 7.—*Nitrogen partition in the leaves of the different strains of Oenothera.*

Key No.	Name of strain.	Characteristic of strain.	Total nitrogen.	Water-soluble nitrogen.	Protein nitrogen.	Free amino-acid nitrogen.	Nitrate nitrogen.	Ammonia nitrogen.	Water-soluble nitrogen.				
									Acid amide and ammonia nitrogen.	Humin nitrogen.	Basic nitrogen.	Non-basic nitrogen.	Peptide nitrogen.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	2.54	0.427	2.09	0.235	0.069	0.055	0.162	0.151	0.092	0.022	0.106
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	3.03	0.607	2.46	0.311	0.087	0.076	0.127	0.188	0.121	0.171	0.020
3b	*Oe. pratincola* hyb. *immunis*	Resistant	2.29	0.709	1.55	0.421	0.132	0.105	0.254	0.194	0.109	0.152	0.00
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	3.08	1.06	2.03	0.588	0.188	0.137	0.327	0.219	0.107	0.407	0.067
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis.*	Resistant	2.45	0.575	1.97	0.351	0.138	0.031	0.168	0.185	0.095	0.127	0.056
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens.*	Susceptible	2.81	0.848	1.99	0.446	0.154	0.037	0.213	0.188	0.118	0.329	0.00
17b	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.*	do	2.68	0.667	2.08	0.537	0.157	0.093	0.218	0.177	0.091	0.181	0.014
18b	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	2.81	0.757	2.11	0.433	0.147	0.093	0.244	0.169	0.112	0.232	0.025
29a	*Oe. pratincola* hyb. *immunis*	Resistant	2.54	0.740	1.82	0.482	0.115	0.098	0.215	0.152	0.071	0.301	0.00
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	2.95	0.933	2.09	0.739	0.166	0.117	0.277	0.193	0.098	0.365	0.00
35	*Oe. "biennis Chicago"*	do	2.91	1.06	2.00	0.626	0.190	0.116	0.318	0.123	0.127	0.492	0.133

In view of the concordant results obtained from all the possible crosses and elementary species, it can be stated that susceptible plants are characterized by having a higher total nitrogen than closely related resistant ones.

Water-soluble nitrogen.—To determine which forms of nitrogen are responsible for the difference in total nitrogen, the nitrogen distribution was determined for four pairs of reciprocal hybrids and three elementary species. It is to be noted that the figures for acid amide and ammonia, humin, and basic and nonbasic nitrogen, given in Tables 7 and 8, were obtained after acid hydrolysis of the water-soluble extract, hence they account for the nitrogen from the water-soluble portion only. Results in Table 7 indicate that: (*a*) in the three pairs of reciprocal hybrids which consist of resistant and susceptible members (key Nos. 1b–9a), the water-soluble nitrogen as a whole and its amino acid and nonbasic nitrogen compounds are higher in the susceptible hybrids than in their resistant reciprocals. The nitrate nitrogen in the susceptible hybrids is somewhat higher than in the resistant ones but the difference is not sufficient to be considered significant. As for the protein nitrogen, in the first two pairs (key Nos. 1b–4b) the susceptible hybrids contain higher protein nitrogen than the resistant ones, but in the third pair (key Nos. 8a–9a) their protein nitrogen is about the same. Other forms of nitrogen do not show concordant results. (*b*) In the fourth pair of reciprocal hybrids, in which both members are susceptible (key Nos. 17b–18b), the water-soluble nitrogen is high and the percentage of the different forms of nitrogen in the water-soluble fraction of both strains does not vary considerably. (*c*) The resistant parent shows the same characteristic features, when compared with the susceptible parent, as its resistant offspring; namely, low water-soluble nitrogen, amino acid, and nonbasic nitrogen. Conversely, the susceptible parent and its susceptible progeny resemble each other in having high water-soluble nitrogen, amino acid, and nonbasic nitrogen.

When the results of the nitrogen distribution, shown in Table 7, are expressed as percentage of the total nitrogen of the leaves, it can be seen that the water-soluble nitrogen is also higher in the susceptible strains than in the resistant ones. The two main components of the water-soluble nitrogen which account for this difference are the free amino acid and the nonbasic nitrogen. For the protein nitrogen, the reverse is found to be the case. (See Table 8.)

TABLE 8.—*Nitrogen partition in the leaves of the different strains of Oenothera.*

[Data expressed as percentage of the total nitrogen of the leaves.]

Key No.	Name of strain.	Characteristic of strain.	Water-soluble nitrogen.	Protein nitrogen.	Free amino-acid nitrogen.	Nitrate nitrogen.	Ammonia nitrogen.	Water-soluble nitrogen.				
								Acid amide and ammonia nitrogen	Humin nitrogen.	Basic nitrogen.	Non-basic nitrogen.	Peptide nitrogen.
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	16.81	82.28	9.25	2.72	2.17	6.38	5.94	3.62	0.866	4.17
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	20.03	81.19	10.26	2.87	2.51	4.19	6.21	3.99	5.64	0.660
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis.*	Resistant	23.47	80.41	14.33	5.63	1.27	6.86	7.55	3.88	5.18	2.29
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens.*	Susceptible	30.18	70.82	15.87	5.48	1.32	7.58	6.69	4.20	11.71	0.00
3b	*Oe pratincola* hyb. *immunis*	Resistant	30.96	67.69	18.39	5.76	4.59	11.09	8.47	4.76	6.64	0.00
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	34.42	65.94	19.09	6.10	4.45	10.79	7.11	3.47	13.21	2.18
17b	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.*	do	24.89	77.61	10.04	5.86	3.47	8.13	6.60	3.40	6.75	0.522
18b	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago".*	do	26.94	76.09	15.41	5.23	3.31	8.68	6.01	3.99	8.26	0.889
29a	*Oe. pratincola* hyb. *immunis*	Resistant	29.13	71.65	18.98	4.53	3.86	8.50	5.98	2.80	11.85	0.00
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	31.63	70.85	25.05	5.63	3.97	9.39	6.54	3.32	12.37	0.00
35	*Oe. "biennis Chicago"*	do	36.43	68.73	21.51	6.53	3.99	10.93	4.23	4.36	16.91	4.57

Protein nitrogen.—In order to determine the nitrogen partition in the protein, the leaf powder was treated with strong hydrochloric acid and boiled under a reflux condenser for several hours. After hydrolysis was complete, the nitrogen distribution was determined, according to the methods of Hausmann as adopted by Jodidi and Moulton (23) in their work with spinach. The results of the experiments are shown in Tables 9 and 10.

TABLE 9.—*Nitrogen partition in the leaves of different strains of Oenothera after acid hydrolysis.*

Key No.	Name of strain.	Characteristic of strain.	Acid amide and ammonia nitrogen.	Humin nitrogen.	Basic nitrogen.	Non-basic nitrogen.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	0.254	0.237	0.398	1.651
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	0.370	0.230	0.499	1.931
3b	*Oe. pratincola* hyb. *immunis*	Resistant	0.384	0.218	0.334	1.354
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	0.556	0.249	0.476	1.799
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.	Resistant	0.307	0.226	0.379	1.538
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*.	Susceptible	0.372	0.213	0.397	1.828
13b	*Oe. mississippiensis* × *Oe. cinerescens*.	do	0.467	0.251	0.379	1.593
14b	*Oe. cinerescens* × *Oe. mississippiensis* (metaclinic hybrid).	do	0.466	0.196	0.360	1.618
17b	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*.	do	0.404	0.257	0.404	1.615
18b	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"*.	do	0.371	0.258	0.417	1.764
29a	*Oe. pratincola* hyb. *immunis*	Resistant	0.342	0.180	0.436	1.582
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	0.465	0.255	0.489	1.741
35	*Oe. "biennis Chicago"*	do	0.434	0.160	0.525	1.791

Table 9 shows that, in the first two pairs of reciprocal hybrids (key Nos. 1b–4b), the acid amide and ammonia nitrogen and the basic nitrogen are higher in the susceptible hybrids than in their resistant reciprocals. In the third pair (key Nos. 8a–9a), however, this difference is not shown to a great extent. With respect to the nonbasic nitrogen, the susceptible hybrids were found to have a higher percentage. The case of the fourth pair, where *Oe. mississippiensis* and *Oe. cinerescens* are the parents (key Nos. 13b–14b), is interesting. One of the members of the pair is a metaclinic hybrid and, as far as

their reaction to mildew is concerned, both are represented as having the same zygotic composition. Their total nitrogen has already been found to be practically the same (Table 6) and here the nitrogen distribution differs to but a slight degree. The fifth pair of reciprocal hybrids (key Nos. 17b and 18b), both members being susceptible, have high acid amide and ammonia nitrogen, and basic and nonbasic nitrogen. Furthermore, the difference between them is slight. Of the parent species (key Nos. 29a-35), the susceptible ones have higher acid amide and ammonia, basic, and nonbasic nitrogen than the resistant one.

TABLE 10.—*Nitrogen partition in the leaves of different strains of Oenothera after acid hydrolysis.*

[Data expressed as percentage of the total nitrogen of the leaves.]

Key No.	Name of strain.	Characteristic of strain.	Acid amide and ammonia nitrogen.	Humin nitrogen.	Basic nitrogen.	Nonbasic nitrogen.
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	10.00	9.33	15.67	65.00
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	12.21	7.59	16.47	63.73
3b	*Oe. pratincola* hyb. *immunis*	Resistant	16.77	9.52	14.59	59.12
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	18.05	8.08	15.45	58.40
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.	Resistant	12.53	9.22	15.47	62.78
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*.	Susceptible	13.24	7.58	14.13	65.05
13b	*Oe. mississippiensis* × *Oe. cinerescens*.	do	17.36	9.33	14.09	59.22
14b	*Oe. cinerescens* × *Oe. mississippiensis* (metaclinic hybrid).	do	17.65	7.42	13.64	61.29
17b	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*.	do	14.38	9.15	14.38	57.47
18b	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	13.84	9.63	15.55	65.82
29a	*Oe. pratincola* hyb. *immunis*	Resistant	13.46	7.09	17.17	62.28
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	15.76	8.64	16.58	59.01
35	*Oe. "biennis Chicago"*	do	14.91	5.50	18.04	61.54

Table 10 shows that the total nitrogen of all the susceptible strains is higher than that of the resistant strains in acid amide and ammonia nitrogen. On the other hand, the total nitrogen of the resistant strains is higher in humin nitrogen than that of the susceptible ones. As for the other nitrogen groups, the results are not concordant.

The nitrogen distribution after acid hydrolysis includes both the nitrogen from the insoluble portion and from the water-soluble portion. Since the nitrogen distribution in the water-soluble portion has also been determined, the different forms of nitrogen corresponding to the water-insoluble or protein portion can be found approximately by subtraction. In the water-insoluble portion, Table 11, it can be noticed that the acid amide and ammonia nitrogen, as well as the nonbasic nitrogen, are higher in the susceptible strains than in the resistant ones. An exception occurs in *Oe. "biennis Chicago,"* one of the parent species, where the acid amide and ammonia nitrogen and the nonbasic nitrogen are somewhat low. However, when these nitrogen groups of the water-insoluble portions are expressed in percentages of the total nitrogen of the leaves, Table 12, the only significant difference that can be noticed is in the nonbasic nitrogen. With respect to this group, the resistant strains are higher than the susceptible. With the exception of *Oe. pratincola* hyb. *immunis*, a parent strain (key No. 29a), the humin nitrogen is also higher in the resistant strains.

TABLE 11.—*Nitrogen partition in the water-insoluble portion of the leaves of different strains of Oenothera, after acid hydrolysis.*

Key No.	Name of strain.	Characteristic of strain.	Acid amide and ammonia nitrogen.	Humin nitrogen.	Basic nitrogen.	Nonbasic nitrogen.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	0.092	0.086	0.306	1.629
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	0.243	0.042	0.378	1.760
3b	*Oe. pratincola* hyb. *immunis*	Resistant	0.130	0.024	0.225	1.202
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	0.229	0.030	0.369	1.392
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.	Resistant	0.139	0.041	0.284	1.411
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*.	Susceptible	0.159	0.025	0.279	1.499
17b	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*.	do	0.186	0.080	0.313	1.434
18b	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	0.127	0.089	0.305	1.532
29a	*Oe. pratincola* hyb. *immunis*	Resistant	0.126	0.028	0.385	1.281
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	0.188	0.062	0.391	1.376
35	*Oe. "biennis Chicago"*	do	0.116	0.037	0.398	1.299

TABLE 12.—*Nitrogen partition in the water-insoluble portion of the leaves of different strains of Oenothera, after acid hydrolysis.*

[Data expressed as percentage of the total nitrogen of the leaves.]

Key No.	Name of strain.	Characteristic of strain.	Acid amide and ammonia nitrogen.	Humin nitrogen.	Basic nitrogen.	Non-basic nitrogen.
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	3.62	3.39	12.02	64.13
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	8.02	1.78	12.48	58.09
3b	*Oe. pratincola* hyb. *immunis*	Resistant	5.68	1.05	9.83	52.48
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	7.26	0.97	11.98	45.19
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.	Resistant	5.67	1.67	12.59	37.60
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*.	Susceptible	5.66	0.89	9.93	53.34
17b	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*.	do	6.25	2.55	10.98	50.72
18b	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	5.16	3.62	11.56	57.56
29a	*Oe. pratincola* hyb. *immunis*	Resistant	4.96	1.11	14.37	50.43
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	6.37	2.11	13.26	46.64
35	*Oe. "biennis Chicago"*	do	3.98	1.27	13.68	44.63

For the purpose of obtaining some information relative to the nitrogen distribution in the leaves of resistant and susceptible strains when they are approaching senescence and the mildew is no longer multiplying, the nitrogen distribution of three pairs of reciprocal hybrids collected in 1920 was determined. The results are shown in Tables 13 to 16. Only two concordant results are indicated in Tables 13 and 14. These are for the free amino acid nitrogen and the protein nitrogen. The resistant hybrids of the first two pairs contain more free amino acid than their corresponding reciprocals. On the other hand, the protein nitrogen of the susceptible hybrids is a great deal higher than that of the resistant ones. The difference in the free amino acid, however, loses its significance, since the last pair, in which both reciprocal hybrids are susceptible, is also found to vary in amino acid nitrogen. Therefore, the cause of the consistent difference of the total nitrogen found in the resistant and susceptible strains must be sought in the protein group. From Table 15, which shows the nitrogen distribution after acid hydrolysis, it is seen that the nitrogen from the basic and nonbasic units is higher in the susceptible strains than in the resistant. The humin nitrogen,

TABLE 13.—*Nitrogen partition in the leaves of different strains of Oenothera during the period when the leaves show signs of chlorophyll degradation (cultures of 1920).*

Key No.	Name of strain.	Characteristic of strain.	Total nitrogen.	Water-soluble nitrogen.	Protein nitrogen.	Free amino acid nitrogen.	Ammonia nitrogen.	Nitrate nitrogen.	Water-soluble nitrogen.			
									Acid amide and ammonia nitrogen.	Humin nitrogen.	Basic nitrogen.	Nonbasic nitrogen.
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	2.560	0.627	1.965	0.423	0.089	0.129	0.322	0.081	0.147	0.077
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	3.385	0.389	3.045	0.263	0.031	0.080	0.128	0.070	0.089	0.096
3	*Oe. pratincola* hyb. *immunis*	Resistant	2.585	0.277	2.254	0.185	0.017	0.031	0.058	0.020	0.056	0.143
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	3.547	0.406	3.150	0.130	0.044	0.073	0.092	0.057	0.071	0.176
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*.	do	3.129	0.300	2.809	0.166	0.020	0.017	0.053	0.029	0.050	0.168
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	3.969	0.741	3.185	0.467	0.036	0.081	0.207	0.032	0.075	0.427

Data expressed as percentage of moisture-free leaves.

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TABLE 14.—*Nitrogen partition in the leaves of different strains of Oenothera during the period when the leaves show signs of chlorophyll degradation (cultures of 1920).*

[Data expressed as percentage of the total nitrogen of the leaves.]

Key No.	Name of strain.	Characteristic of strain.	Water-soluble nitrogen.	Protein nitrogen.	Free amino acid nitrogen.	Nitrate nitrogen.	Ammonia nitrogen.	Water-soluble nitrogen.			
								Acid amide and ammonia nitrogen.	Humin nitrogen.	Basic nitrogen.	Non-basic nitrogen.
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	24.49	76.75	16.52	5.04	3.48	12.58	3.16	5.74	3.01
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	11.49	89.95	7.77	2.36	0.92	3.78	2.24	2.63	2.84
3	*Oe. pratincola* hyb. *immunis*	Resistant	10.72	87.20	7.16	1.20	0.66	2.24	0.77	2.17	5.53
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	11.45	88.81	3.67	2.06	1.24	2.59	1.61	2.00	4.96
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.*	do	9.59	89.77	5.31	0.54	0.64	1.69	0.93	1.60	5.36
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	18.67	80.25	11.77	2.04	0.91	5.22	0.81	1.89	10.75

TABLE 15.—*Nitrogen partition in the leaves of different strains of Oenothera during the period when the leaves show signs of chlorophyll degradation (cultures of 1920).*

[After acid hydrolysis.]

Key No.	Name of strain.	Characteristic of strain.	Acid amide nitrogen in—		Humin nitrogen in—		Basic nitrogen in—		Nonbasic nitrogen in—	
			Moisture-free leaves.	Total nitrogen of leaves.	Moisture-free leaves.	Total nitrogen of leaves.	Moisture-free leaves.	Total nitrogen of leaves.	Moisture-free leaves.	Total nitrogen of leaves.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	0.492	19.22	0.215	8.398	0.395	15.43	1.458	56.95
2	Mildewed *Oe. pratincola* (a segregate from species hybrid.)	Susceptible	0.377	11.14	0.119	3.516	0.527	15.57	2.362	69.78
3	*Oe. pratincola* hyb. *immunis*	Resistant	0.216	8.356	0.315	12.19	0.492	19.03	1.562	60.42
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	0.339	9.557	0.189	5.328	0.722	20.36	2.297	64.76
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.*	do	0.309	9.875	0.177	5.657	0.697	22.28	1.946	62.19
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	0.458	11.54	0.241	6.072	0.793	19.98	2.477	62.41

TABLE 16.—*Nitrogen partition in the water-insoluble portion of the leaves of different strains of Oenothera during the period when the leaves show signs of chlorophyll degradation (cultures of 1920).*

Key No.	Name of strain.	Characteristic of strain.	Acid amide nitrogen in—		Humin nitrogen in—		Basic nitrogen in—		Nonbasic nitrogen in—	
			Moisture-free leaves.	Total nitrogen of leaves.	Moisture-free leaves.	Total nitrogen of leaves.	Moisture-free leaves.	Total nitrogen of leaves.	Moisture-free leaves.	Total nitrogen of leaves.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	0.170	6.67	0.134	5.238	0.248	9.690	1.381	53.94
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	0.249	7.36	0.043	1.276	0.438	12.940	2.266	66.94
3	*Oe. pratincola* hyb. *immunis*	Resistant	0.158	6.116	0.295	11.42	0.436	16.86	1.419	54.89
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	0.247	6.967	0.132	3.718	0.651	18.36	2.121	59.80
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.*	do	0.256	8.185	0.148	4.727	0.647	20.68	1.778	56.83
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	0.251	6.32	0.209	5.262	0.718	18.09	2.050	51.65

however, is higher in the resistant hybrids than in their corresponding susceptible reciprocals. These differences are not shown by the last pair, where both reciprocal hybrids are susceptible. Furthermore, as can be seen from Table 16, these differences are traceable to the forms of nitrogen in the water-insoluble portion, which is made up mostly of protein. It seems, therefore, that the water-soluble nitrogenous constituents of the leaves are the ones whose variations show consistent correlations with the occurrence of mildew.

The results of the nitrogen distribution, as a whole, lead to the following conclusions:

1. The main difference between resistant and susceptible strains lies in the water-soluble nitrogen, the susceptible strains having a considerably higher value than the resistant.
2. The forms of combination that are responsible for the difference are the free amino acid and the nonbasic nitrogen, the susceptible plants being characterized by having higher values than the resistant plants.
3. The total nitrogen of the resistant strains has a higher proportion of protein than that of the susceptible ones, the difference being due to the protein nitrogen of nonbasic character.
4. Strains which have the same genetic composition with respect to their reactions to mildew have also practically the same nitrogen distribution.
5. In pairs of reciprocal hybrids both of which are susceptible, the water-soluble nitrogen and the free amino acid are comparatively high and there is not much difference in the distribution.
6. The parent species exhibit the same characteristic differences as the contrasting reciprocal hybrids derived from them.

THE CARBOHYDRATES

The significant differences in the sugar content of the *Oenothera* strains are shown in Table 17. Other carbohydrates do not show consistent correlations with mildew resistance. When the plants are approaching maturity and the mildew on the leaves of the susceptible plants is no longer growing, the amounts of sugar in the resistant and in the susceptible strains do not differ considerably. Furthermore, the slight variation observed is not always in the same direction. In the majority of cases, the sugar content of the resistant strains is higher than that of the susceptible ones. This observation is based upon the analyses of the samples collected in 1920. However,

when the same strains were grown in the following years and their leaves collected at the time when the plants were in active growth, at which time the mildew was also most active, the results of the sugar determinations were strikingly correlated with infection.

The figures for 1921 and 1922 in Table 17 show that the total sugar by acid hydrolysis and the free reducing sugar are higher in the resistant strains than in the susceptible ones. Moreover, in the pair in which both reciprocal hybrids are susceptible (key Nos. 17b–18b), the amount of sugar is extremely low, whereas senescent leaves of the same pair had a high sugar content in 1920. It was thought at first that this variation might be ascribed to the fact that in the susceptible strains the mildew is continually using a part of the sugar. It also seemed possible that in the leaves covered with mildew photosynthesis would be retarded. If these factors operated, one would expect to find more sugar in the full-grown leaves before infection than after. With this point in mind, the sugar content was determined in the leaves of different strains of *Oenothera* collected at two different periods of growth. The results in Table 18 are not in accord with the expectation, for it can be seen that in the case of the resistant strains the sugar content for the two periods is comparatively high, while in the susceptible strains the amount of sugar is even less in the leaves collected before infection than in those collected after infection. When the leaves of the resistant strains are compared with those of the susceptible ones of the same age, the resistant strains are characterized by higher sugar content than the susceptible ones. The results obtained suggest that the difference in sugar content must be due to the constitutional peculiarities of the resistant and the susceptible plants.

Still another possibility to be considered is the rate of translocation of the sugar from the leaves to other parts of the plants, for it might be that the movement of materials proceeds at a greater rate in the vegetatively more vigorous susceptible plants than in the resistant plants. If this supposition were correct, one would have to view the low sugar of the luxuriant susceptible plants, in contrast to the high sugar of the smaller, less rapidly growing resistant plants, as due to the removal of sugar to growing points and to ripening fruits. After all parts of the plant had come to maturity, the differ-

TABLE 17.—*Determination of carbohydrates in the leaves of different strains of Oenothera.*

[Percentage of dry weight.]

Key No.	Name of strain.	Characteristic of strain.	Year of collection.	Total sugar by acid hydrolysis.	Free reducing sugar.	Reducing sugar freed by hydrolysis.	Pentosans.	Starch.
1	Resistant *Oe. pratincola* (a segregate from species hybrid)	Resistant	1920	8.34	6.14	2.20	7.21	4.65
1a			1921	9.65	8.62	1.03	7.59	5.10
1b			1922	13.82	12.73	1.09		
2	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	1920	8.17	6.66	1.51	6.39	4.37
2a			1921	5.18	4.55	0.63	6.99	4.80
2b			1922	8.79	8.43	0.36		
3	*Oe. pratincola* hyb. *immunis*	Resistant	1920	12.68	7.44	5.24	7.80	5.03
3a			1921	6.02	5.05	0.97	7.84	5.68
3b			1922	10.62	10.23	0.39		
4	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	1920	9.42	6.93	2.49	6.55	4.87
4a			1921	2.79	2.17	0.62	7.40	4.32
4b			1922	7.58	7.20	0.38		
5	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*	Resistant	1920	7.81	6.76	1.05	7.45	4.95
5a			1921	4.73	4.23	0.50	8.10	3.95
6	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*	Susceptible	1920	8.28	6.97	1.31	6.50	4.78
6a			1921	4.34	4.18	0.16	7.00	3.48
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant	1921	7.33	6.93	0.40	8.14	5.23
8a			1922	9.85	8.41	1.44		
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible	1921	6.85	6.53	0.31	6.63	5.10
9a			1922	6.40	5.79	0.61		
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*	do	1920	10.37	7.44	2.93	6.80	4.32
17b			1922	2.66	1.82	0.84		
18	*Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago"*	do	1920	10.72	8.75	1.97	6.88	4.57
18b			1922	2.30	1.97	0.33		
29a	*Oe. pratincola* hyb. *immunis*	Resistant	1922	9.90	9.58	0.42	8.36	
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	1922	8.27	7.62	0.65	8.24	
35	*Oe. "biennis Chicago"*	do	1922	6.25	5.34	0.91	8.06	

TABLE 18.—*Determination of sugar in the leaves of different strains of Oenothera collected at two different periods of growth during 1922.*

Key No.	Name of strain.	Date of collection.	Condition of strain during collection.	Total sugar by acid hydrolysis.	Free reducing sugar.	Reducing sugar freed by hydrolysis.
				Per cent.	*Per cent.*	*Per cent.*
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	July 19	No mildew	11.37	10.51	0.86
		August 30	do	13.82	12.73	1.09
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	July 19	do	8.79	8.43	0.36
		August 30	Mildew	10.56	9.37	1.19
3b	*Oe. pratincola* hyb. *immunis*	July 5	No mildew	10.62	10.23	0.39
		August 31	do	10.64	10.04	0.60
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	July 5	do	7.58	7.20	0.38
		August 31	Mildew	7.58	6.75	0.83
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	July 5	No mildew	11.20	10.63	0.57
		August 30	do	9.85	8.41	1.44
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	July 5	do	3.71	2.99	0.72
		August 30	Mildew	6.40	5.79	0.61
29a	*Oe. pratincola* hyb. *immunis*	July 6	No mildew	17.32	16.66	0.66
		August 28	do	9.90	9.58	0.42
34	*Oe. pratincola* hyb. *rubricalyx*	July 5	do	8.16	7.56	0.60
		August 28	Mildew	8.27	7.62	0.65
35	*Oe. "biennis Chicago"*	July 5	No mildew	8.10	7.70	0.40
		August 28	Mildew	6.25	5.34	0.91

ence should cease to exist. The results are not inconsistent with this hypothesis which, to be proved, would require elaborate experimentation. In any event, it would remain true that the low sugar of the susceptible strains is a constitutional peculiarity, and not due to modification by the mildew.

With respect to the pentosan in the leaves, Table 17 seems to show that the resistant strains contain more than the susceptible ones. However, when the determination was extended to other strains (see Table 19), it was found that pairs of reciprocal hybrids in which both members are susceptible may also show a high pentosan content. Consequently, the results do not permit any possible interpretation. One significant feature of the pentosan determination, however, is that strains similar in genetic composition also have nearly the same percentage of pentosan. This can be noticed in crosses between *Oe. mississippiensis* and *Oe. cinerescens* where one of the hybrids is a metaclinic (key Nos. 13a and 14).

The amount of starch in the leaves of the resistant and the susceptible strains does not differ considerably.

TABLE 19.—*Determination of pentosans in the leaves of different strains of Oenothera.*

Key No.	Name of strain.	Characteristic of strain.	Pentosans in 1920.	Pentosans in 1921.
			Per cent.	*Per cent.*
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	7.21	
1a				7.59
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	6.39	
2a				6.99
3	*Oe. pratincola* hyb. *immunis*	Resistant	7.80	
3a				7.84
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	6.55	
4a				7.40
5	*Oe. missisippiensis* × *Oe. pratincola* hyb. *immunis*.	Resistant	7.45	
5a				8.10
6	*Oe. pratincola* hyb. *immunis* × *Oe. missisippiensis*.	Susceptible	6.50	
6a				7.00
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.	Resistant		8.14
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*.	Susceptible		6.63
10	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*.	Resistant	7.56	
11	*Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."*	Susceptible	6.91	
13a	*Oe. missisippiensis* × *Oe. cinerescens*	do		7.97
14	*Oe. cinerescens* × *Oe. missisippiensis*, meta-clinic hybrid.	do		8.29
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*.	do	6.80	
17a				7.30
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	6.88	
18a				7.24
19a	*Oe. missisippiensis* × *Oe. "biennis Chicago."*	do		6.80
21a	*Oe. "biennis Chicago" Oe. missisippiensis*	do		7.61
22a	*Oe. missisippiensis* × *Oe. pratincola* hyb. *rubricalyx*.	do		8.11
23a	*Oe. pratincola* hyb. *rubricalyx* × *Oe. missisippiensis*.	do		6.32
24	*Oe. pratincola* mut. *nitidissima*	Resistant		7.82
24a	do	do		7.90
25	do	Susceptible		7.22
25a	do	do		7.18
29a	*Oe. pratincola* hyb. *immunis*	Resistant		8.86
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible		8.24
35	*Oe. "biennis Chicago"*	do		8.06

THE TANNIN

In the determination of the sugar, it was noticed that the amount of Fehling's solution reduced by the unclarified extract of the leaves was much higher than that for the same extract

after it was clarified with lead subacetate solution. Another striking thing was that the unclarified extract of the resistant strains showed higher reduction than that of the susceptible ones. Table 20 shows the results alluded to. It was obvious that there must be in the extract some substance other than sugar that possessed the power of reducing Fehling's solution and that differed from sugar in being precipitated by the lead subacetate. It could not be a glucoside of the usual type, for the high reduction of Fehling's solution was noticed in the extract before hydrolysis. Moreover, the percentage of reducing sugar freed by hydrolysis does not differ very much in the clarified and the unclarified extracts. The extract when tested with ferric chloride solution gave a deep blackish blue color, suggesting that tannin must be the other substance in the extract which reduced the Fehling's solution and was precipitated with lead subacetate. Accordingly, a tannin determination was carried out for a number of strains and the results in Table 21 are in accordance with the expectation. Some characteristic features that are shown in the table are:

1. In pairs of reciprocal hybrids, the resistant one contains a much higher percentage of tannin than the susceptible one, the difference varying from 50 to 150 per cent.

2. Those elementary species which are resistant have higher tannin content than those which are susceptible, the only exception being *Oe. "biennis Chicago,"* a susceptible species in which the tannin is rather high.

3. Of pairs in which both reciprocal hybrids are susceptible, the tannin content is correspondingly low.

4. The metaclinic hybrid *Oe. cinerescens* × *Oe. mississippiensis* and its reciprocal are found to contain almost the same amount of tannin.

5. Of the two strains of *Oe. pratincola* mut. *nitidissima* the resistant one is higher in tannin than the susceptible one.

THE CRUDE FIBER

The determinations of crude fiber that are given in Table 22 indicate that the resistant strains have higher crude fiber than the susceptible ones.

THE WATER-SOLUBLE ACIDS

There is a much greater amount of water-soluble acid in the leaves of the resistant hybrids than in the leaves of those which are susceptible (see Table 23). In the elementary species, the resistant ones are also conspicuously higher in total acid.

TABLE 20.—*Reducing sugar in the leaves of the different strains of Oenothera.*

[Percentage of dry weight.]

Key No.	Name of strain.	Characteristic of strain.	Year of collection.	Clarified solution.			Unclarified solution.		
				Reducing sugar before inversion.	Reducing sugar after inversion.	Reducing sugar freed by hydrolysis.	Reducing sugar before inversion.	Reducing sugar after inversion.	Reducing sugar freed by hydrolysis.
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	1920	6.14	8.34	2.20	18.27	20.63	2.36
1a			1921	8.62	9.65	1.03	21.70	22.64	0.94
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	1920	6.66	8.17	1.51	13.60	15.95	2.35
2a			1921	4.55	5.18	0.63	12.22	13.56	1.34
3	*Oe. pratincola* hyb. *immunis*	Resistant	1920	7.44	12.68	5.24	18.05	23.07	5.02
3a			1921	5.05	6.02	0.97	18.63	20.81	2.18
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	1920	6.93	9.42	2.49	15.69	20.18	4.49
4a			1921	2.17	2.79	0.62	10.27	11.29	1.02
5	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis.*	Resistant	1920	6.76	7.81	1.05	16.41	18.84	2.43
6	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis.*	Susceptible	1920	6.97	8.28	1.31	13.47	15.49	2.07
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant	1921	6.93	7.33	0.40	15.03	16.87	1.84
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible	1921	6.53	6.85	0.31	11.46	12.90	1.44
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.*	do	1920	7.44	10.37	2.93	21.24	24.04	2.80
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago".*	do	1920	8.75	10.72	1.97	18.46	20.35	1.89

In the case of the strains that have the same zygotic composition as far as their reaction with the mildew is concerned, the percentage of the water-soluble acid does not differ to a great extent. This is shown by the metaclinic hybrid *Oe. cinerescens* × *Oe. mississippiensis* and its reciprocal. The parent species exhibit the same characteristic as their corresponding hybrids. For example, the water-soluble content in the resistant *Oe. pratincola* hyb. *immunis* is almost twice as high as in *Oe. pratincola* hyb. *rubricalyx*, a susceptible parent.

TABLE 21.—*Percentage of tannin in the leaves of different strains of Oenothera.*

[Expressed in terms of gallotanic acid.]

Key No.	Name of strain.	Characteristic of strain.	Tannin in 1920.	Tannin in 1921.	Tannin in 1922.
			Per cent.	*Per cent.*	*Per cent.*
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	13.24		
1a				10.08	
1b					9.87
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	5.66		
2a				3.47	
2b					5.05
3	*Oe. pratincola* hyb. *immunis*	Resistant	8.96		
3a				9.79	
3b					10.22
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	5.41		
4a				5.43	
4b					6.22
5	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*.	Resistant	6.87		
5a				6.58	
6	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*.	Susceptible	3.83		
6a				4.49	
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.	Resistant		5.49	
8a					8.30
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*.	Susceptible		2.22	
9a					3.58
10	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*.	Resistant	9.27		
11	*Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."*	Susceptible	4.41		
13a	*Oe. mississippiensis* × *Oe. cinerescens*	do		3.94	
13b					5.83
14	*Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid.	do		3.54	
14a					5.19
17	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*.	do	9.14		
17a				6.82	
17b					9.42
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*	do	6.30		
18a				8.52	
18b					9.84
22	*Oe. mississippiensis* × *Oe. pratincola* hyb. *rubricalyx*.	do	6.09		
22a				4.99	

TABLE 21.—*Percentage of tannin in the leaves of different strains of Oenothera*—Continued.

Key No.	Name of strain.	Characteristic of strain.	Tannin in 1920.	Tannin in 1921.	Tannin in 1922.
			Per cent.	*Per cent.*	*Per cent.*
23	*Oe. pratincola* hyb. *rubricalyx* × *Oe. mississippiensis.*	Susceptible	6.77		
23a				5.35	
24	*Oe. pratincola* mut. *nitidissima*	Resistant		10.96	
24a	do	do		11.12	
25	do	Susceptible		8.66	
26	*Oe. numismatica*	Resistant		12.74	
27	*Oe. pratincola* mut. *simulans*	do		10.05	
28	*Oe. pratincola* mut. *simulans rubricalyx*	Susceptible		8.68	
29	*Oe. pratincola* hyb. *immunis*	Resistant		9.95	
29a					10.48
30	*Oe. reynoldsii* × *Oe. pratincola*	do		8.82	
31	*Oe. pratincola* (susceptible)	Susceptible		6.19	
32	*Oe. mississippiensis*	do		6.99	
33	*Oe. reynoldsii*	Resistant		11.03	
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible			5.10
35	*Oe.* "biennis Chicago"				10.85

TABLE 22.—*Determination of crude fiber in the leaves of different strains of Oenothera.*

Key No.	Name of strain.	Characteristic of strain.	Crude fiber in 1920.	Crude fiber in 1921.
			Per cent.	*Per cent.*
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	7.77	
1a				6.02
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	5.60	
2a				5.85
3	*Oe. pratincola* hyb. *immunis*	Resistant	6.41	
3a				6.49
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	5.43	
4a				6.15
5	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis.*	Resistant	7.26	
5a				7.45
6	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis.*	Susceptible	6.39	
6a				6.80
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant		7.29
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible		6.19
24	*Oe. pratincola* mut. *nitidissima*	Resistant		7.12
24a	do	do		7.33
25	do	Susceptible		6.53
25a	do	do		6.41

THE TOTAL ASH

The results of the total ash determination in Table 24 indicate that, with a few exceptions, the susceptible strains contain much

more total ash than the resistant ones. Exceptions were noted in hybrids resulting from *Oe. mississippiensis* and *Oe. pratincola* hyb. *immunis*, *Oe. "biennis Chicago"* and *Oe. pratincola* hyb. *immunis*, and *Oe. cinerescens* and *Oe. mississippiensis*. However, in these exceptional cases the percentage of total ash in the resistant hybrids is only slightly higher than in their susceptible reciprocals.

TABLE 23.—*Determination of the water-soluble acid in the leaves of different strains of Oenothera.*

[Expressed in milligrams of sodium hydroxide required to neutralize the acid from 1 gram of moisture-free leaves.]

Key No.	Name of strain.	Characteristic of strain.	1920	1921	1922
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	37.56		
1a				25.21	
1b					21.61
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	25.36		
2a				20.65	
2b					17.24
3	*Oe. pratincola* hyb. *immunis*	Resistant	24.16		
3a				23.28	
3b					25.60
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	23.12		
4a				21.92	
4b					19.28
5	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*.	Resistant	30.04		
5a				20.02	
6	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*.	Susceptible	24.16		
6a				21.96	
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.	Resistant		21.36	
8a					19.52
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*.	Susceptible		13.72	
9a					13.68
10	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*.	Resistant	32.04		
11	*Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."*	Susceptible	25.24		
12	*Oe. cinerescens* × *Oe. mississippiensis*	Resistant	24.64		
13	*Oe. mississippiensis* × *Oe. cinerescens*	Susceptible	23.84		
13a				14.72	
13b					18.64
14	*Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid.	do		13.44	
14a					18.26
24	*Oe. pratincola* mut. *nitidissima*	Resistant		30.40	
24a	do	do		31.64	
25	do	Susceptible		26.72	
25a	do	do		27.15	
29a	*Oe. pratincola* hyb. *immunis*	Resistant			23.85
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible			12.43
35	*Oe. "biennis Chicago"*	do			19.15

THE INORGANIC CONSTITUENTS OF THE ASH

In order to find out the constituents of the ash that differ to a marked degree in the resistant and the susceptible strains, partial ash analyses were made. The results, given in Table 25, reveal that the main difference is found in the calcium and sulphur content. The percentages of these two elements are higher in the leaves of the susceptible strains than in those of the resistant ones. To a small degree the susceptible strains also contain more phosphorus and potassium. When the different constituents are expressed as percentages of the total ash (Table 26), the results for potassium and phosphorus are not concordant, while those for calcium and sulphur are; that is, the total ash of the susceptible strains contains more calcium and sulphur than does that of the resistant ones.

In view of the marked differences noted in the percentage of total nitrogen, total ash, sugar, tannin, and water-soluble acid in the resistant and the susceptible strains, further analyses were made to see if these differences existed in the plants before the susceptible strains were mildewed. These five constituents were, therefore, determined in the leaves of several strains collected at two different periods of growth; one when the susceptible strains were not yet infected, and the other when they were infected. The results in Table 27 show some interesting facts. In the first place, the characteristic chemical differences between the resistant and the susceptible strains are found to be exactly the same, both before and after infection. In fact, the differences are even more pronounced before infection, for the resistant strains are exceedingly high in tannin and water-soluble acid, while the susceptible ones contain a great amount of total ash and total nitrogen. Another point of interest is that the amounts of the total nitrogen, tannin, total ash, and water-soluble acid do not differ much for the two periods in the resistant strains; whereas, in the susceptible, the percentage of these four constituents varies considerably for the two periods, the variation being in such direction as to render their difference from the resistant ones far more significant. The results obtained for the sugar have already been presented separately in Table 18, in connection with the carbohydrates.

EXTENSION OF THE WORK TO OTHER PLANTS

To determine whether the chemical differences existing in the different strains of *Oenothera* are also found in other

TABLE 24.—*Percentage of total ash in the leaves of different strains of Oenothera.*

Key No.	Name of strain.	Characteristic of strain.	Total ash in 1920.	Total ash in 1921.	Total ash in 1922.
			Per cent.	*Per cent.*	*Per cent.*
1	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	11.09		
1a				9.04	
1b					8.77
2	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	13.40		
2a				13.14	
2b					11.16
3	*Oe. pratincola* hyb. *immunis*	Resistant	11.29		
3a				9.57	
3b					10.81
4	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	13.10		
4a				13.28	
4b					13.93
5	*Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*.	Resistant	11.27		
5a				12.81	
6	*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*.	Susceptible	10.16		
6a				12.06	
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.	Resistant		10.65	
8a					11.58
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*.	Susceptible		13.54	
9a					15.03
10	*Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*.	Resistant	11.90		
11	*Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."*	Susceptible	11.49		
12	*Oe. cinerescens* × *Oe. mississippiensis*	Resistant	13.63		
13	*Oe. mississippiensis* × *Oe. cinerescens*	Susceptible	13.49		
13a				13.66	
13b					14.83
14	*Oe. cinerescens* × *Oe. mississippiensis*, meta-clinic hybrid.	do		14.00	
14b					14.68
15	*Oe. pratincola* × *Oe. reynoldsii* (*Oe. pratincola* hyb. *amycosa*).	Resistant	10.84		
16	*Oe. reynoldsii* × *Oe. pratincola*	Susceptible	11.26		
17a	*Oe. "bienni*[8] *Chicago"* × *Oe. pratincola* hyb. *rubricalyx*.	do		11.18	
17b					11.08
18	*Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"*.	do		11.73	
18a					12.96
24	*Oe. pratincola* mut. *nitidissima*	Resistant		10.44	
24a	do	do		9.97	
25	do	Susceptible		11.88	
25a	do	do		11.10	
26	*Oe. numismatica*	Resistant		9.76	
27	*Oe. pratincola* mut. *simulans*	do		10.63	
28	*Oe. pratincola* mut. *simulans rubricalyx*			11.39	
29a	*Oe. pratincola* hyb. *immunis*	Resistant			7.47
31	*Oe. pratincola*	Susceptible		12.94	
32	*Oe. mississippiensis*	do		11.51	
33	*Oe. reynoldsii*	Resistant		10.60	
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible			13.04
35	*Oe. "biennis Chicago"*	do			11.55

TABLE 25.—*Constituents of the ash in the leaves of different strains of Oenothera.*

Key No.	Name of strain.	Characteristic of strain.	Total ash.	Constituents of ash, expressed as percentage of moisture-free leaves.						
				SiO_2	CaO	MgO	K_2O	Na_2O	P_2O_5	SO_3
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	8.77	0.346	3.32	1.04	0.713	0.139	0.425	0.541
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	11.16	0.414	4.51	1.10	1.07	0.134	0.628	0.875
3b	*Oe. pratincola* hyb. *immunis*	Resistant	10.81	0.445	4.24	1.07	1.08	0.088	0.560	0.763
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	13.93	0.549	5.87	1.36	11.36	0.048	0.663	1.067
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis.*	Resistant	11.58	0.325	4.47	1.25	1.21	0.086	0.558	0.867
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens.*	Susceptible	15.03	0.387	6.80	1.39	1.41	0.052	0.688	1.99
29a	*Oe. pratincola* hyb. *immunis*	Resistant	7.47	0.217	2.66	0.677	0.702	0.044	0.391	0.508
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	13.04	0.539	5.01	1.48	1.64	0.177	0.633	1.17

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TABLE 26.—*Constituents of the ash in the leaves of different strains of Oenothera.*

Key No.	Name of strain.	Characteristic of strain.	Expressed as percentage of total ash.						
			SiO_2	CaO	MgO	K_2O	Na_2O	P_2O_5	SO_3
1b	Resistant *Oe. pratincola* (a segregate from species hybrid).	Resistant	3.94	37.81	11.59	8.13	1.59	4.85	6.18
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	3.71	40.45	9.89	9.61	1.20	5.63	7.85
3b	*Oe. pratincola* hyb. *immunis*	Resistant	4.12	39.26	10.35	10.02	0.812	5.18	7.06
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid).	Susceptible	3.94	42.15	9.78	9.74	0.342	4.76	7.65
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant	2.81	38.61	10.76	10.46	0.745	4.82	7.48
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible	2.58	45.25	9.24	9.37	0.348	4.58	13.23
29a	*Oe. pratincola* hyb. *immunis*	Resistant	2.90	35.61	9.07	9.40	0.590	5.24	6.71
34	*Oe. pratincola* hyb. *rubricalyx*	Susceptible	4.13	38.41	11.32	12.54	1.36	4.86	8.96

TABLE 27.—*Total nitrogen, tannin, total ash, and water-soluble acid in the leaves of different strains of Oenothera collected at two different periods of growth during 1922.*

Key No.	Name of strain.	Date of collection.	Condition of strain during collection.	Total nitrogen.	Tannins.	Total ash.	Water-soluble acid in terms of mgm. NaOH.[a]
				Per cent.	*Per cent.*	*Per cent.*	
1b	Resistant *Oe. pratincola* (a segregate from species hybrid)	July 19	No mildew	2.40	10.80	8.21	30.32
		August 30	do	2.54	9.87	8.77	21.61
2b	Mildewed *Oe. pratincola* (a segregate from species hybrid)	July 19	do	3.63	4.30	12.70	21.28
		August 30	Mildew	3.03	5.05	11.16	17.24
3b	*Oe. pratincola* hyb. *immunis*	July 6	No mildew	2.06	10 85	7.54	26.36
		August 30	do	2.29	10.22	10.81	25.60
4b	Mildewed *Oe. pratincola* (a segregate from species hybrid)	July 6	do	3.86	3.98	14.19	17.24
		August 30	Mildew	3.08	6.22	13.93	19.28
8a	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	July 6	No mildew	2.54	9.30	10.19	26.48
		August 30	do	2.45	8.30	11.58	19.52
9a	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	July 6	do	3.94	2.38	16.57	17.76
		August 30	Mildew	2.81	3.58	15.03	13.68
13b	*Oe. mississippiensis* × *Oe. cinerescens*	July 6	No mildew	3.81	5.56	15.12	20.88
		August 30	Mildew	2.69	5.83	14.83	18.64
14a	*Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid	July 6	No mildew	3.67	5.71	14.72	20.80
		August 30	Mildew	2.64	6.19	14.68	18.26
29a	*Oe. pratincola* hyb. *immunis*	July 6	No mildew	2.31	10 69	7.75	25.68
		August 28	do	2.54	10.48	7.47	23.85
34	*Oe. pratincola* hyb. *rubricalyx*	July 6	do	3.84	2.46	14.65	13.96
		August 28	Mildew	2.95	5.10	13.04	12.43
35	*Oe. "biennis Chicago"*	July 5	No mildew	3.49	5.47	12.89	21.20
		August 28	Mildew	2.91	10.85	11.55	19.15

[a] The water-soluble acid is expressed as the number of milligrams of sodium hydroxide required to neutralize the acid from 1 gram of moisture-free leaves.

plants that are infected by other forms of powdery mildew, the determination for total nitrogen, total ash, tannin, and water-soluble acid was extended to *Syringa vulgaris, Desmodium canadensis, Helianthus giganteus,* and *Solidago canadensis,* all of these plants having been found to have resistant and susceptible varieties. The results of the determination are given in Table 28. Except for the total ash and water-soluble acid of the strains of *Helianthus giganteus,* the findings confirm those already found in *Oenothera.* There was little time available for this work, which was undertaken through curiosity and with the knowledge that it could not be carried far. It indicates that the results for *Oenothera* are probably of general applicability, and that any of the foregoing genera, and doubtless many others, would afford material that would be equally interesting from the standpoint of genetics and disease resistance in *Oenothera.*

TABLE 28.—*Total nitrogen, tannin, total ash, and water-soluble acid (in terms of NaOH required for neutralization of acid from 1 gram of moisture-free leaves) in the leaves of some plants which were found during the summer of 1922 to have strains resistant and susceptible to powdery mildew.*

Name of plant.	Characteristic of plant.	Total nitrogen.	Total tannin.	Total ash.	Water-soluble acid mgm. NaOH.
		Per cent.	*Per cent.*	*Per cent.*	
Syringa vulgaris	Highly resistant	1.99	2.52	9.93	13.40
Do	Susceptible	2.68	1.40	12.48	10.08
Desmodium canadensis	Resistant	2.88	4.39	7.32	11.24
Do	Susceptible	2.95	2.06	8.51	10.92
Helianthus giganteus	Resistant	1.98	6.36	17.93	7.16
Do	Susceptible	2.36	5.98	11.57	7.12
Solidago canadensis	Resistant	2.43	0.85	10.62	11.40
Do	Susceptible	2.76	0.003	12.47	8.48

DISCUSSION OF RESULTS

The whole body of experimental data brought forward indicates that the chemical differences between the resistant and the susceptible strains of *Oenothera* are definite hereditary characteristics, and that these chemical characteristics are correlated with the degree of resistance of the various strains to mildew. This statement holds true for the nitrogen distribution, the tannin, the water-soluble acid, and the total ash. Therefore, it is possible, if the analysis of a strain is given, to predict more or less accurately what the reaction to mildew will be. It is not possible, however, to use the percentage of any one constituent as

an unfailing index. When the results of analysis are expressed as percentages it is obvious that, if one constituent is high, other constituents must be correspondingly low. A series of ratios, in which some one constituent is taken as unity, indicates more clearly than does the percentage composition the significance of variation in single constituents. Such ratios, for example, show at a glance whether or not two strains differing in percentage composition have actually the same relative composition except with regard to one constituent. For example, if the total nitrogen content of all strains under consideration is considered as unity, the ratios of the various constituents may be summarized for comparison at a glance, as in Table 29.

In Table 29 the ratios show a correlation or at least a parallelism of only two constituents, namely, total nitrogen and total ash. There seems to be no consistent quantitative interdependence among the other constituents, except that pentosan and water-soluble acid show parallel variation. The ratios with constituents other than total nitrogen taken as unity are shown in Tables 30 to 33.

If the amount of any one constituent alone determined resistance or susceptibility, one might expect to find all the other constituents showing parallel variation such as we find in the case of total nitrogen and total ash, or perhaps nonconcordant variation in several pairs of reciprocals. The former condition might be expected in forms morphologically identical but different physiologically, as indicated by unlike resistance to infection. The latter condition would be expected in morphologically unlike strains as well as in those physiologically unlike, since of course one would expect structural dissimilarities of considerable magnitude to be reflected in some way in the chemical composition. Nothing is more obvious from an examination of the tables, however, than that every resistant strain is higher in tannin and water-soluble acid than the genetically most closely related susceptible strain which may be compared with it. Conversely, the resistant strains are correspondingly low in total nitrogen and ash. The results for sugar are not concordant.

Our results, therefore, fail to point to any single constituent as the basis for resistance, and we are justified in concluding only that, when an *Oenothera* is low in nitrogen and ash and high in tannin and acid, it is a resistant strain; if the situation is reversed, the strain is susceptible. As far as we can draw conclusions from the results, we may look upon resistance as having several or many contributory factors.

TABLE 29.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, total nitrogen being taken as unity.*

Key No.	Name of strain.	Characteristic of strain.	Total nitrogen.	Pentosan.	Total sugar by acid hydrolysis.	Tannin.	Water-soluble acid.	Total ash.
1a	Resistant *Oe. pratincola* (a segregate from species hybrid)	Resistant	100	293 05	372.59	389.19	973.36	349.03
2a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	193.62	143.49	96.12	572.00	363.98
3a	*Oe. pratincola* hyb. *immunis*	Resistant	100	281.00	181.00	350.89	834.40	342.98
4a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	191.21	56.07	140.31	566.39	343.14
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant	100	252.79	227.63	168.94	663.33	330.74
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible	100	181.64	187.67	60.82	375.89	370.96
13a	*Oe. mississippiensis* × *Oe. cinerescens*	do	100	272.01	----------	134.47	502.38	466.62
14	*Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid	do	100	289.86	----------	123.78	469.93	489.51
24	*Oe. pratincola* mut. *nitidissima*	Resistant	100	280.28	----------	392.83	1,089.59	374.19
25	do	Susceptible	100	225.62	----------	274.05	845.55	375.94

TABLE 30.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, total ash being taken as unity.*

Key No.	Name of strain.	Characteristic of strain.	Total ash.	Pentosan.	Total nitrogen.	Total sugar by acid hydrolysis.	Tannin.	Water-soluble acid.
1a	Resistant *Oe. pratincola* (a segregate from species hybrid)	Resistant	100	83.95	28.65	106.74	111.50	278.85
2a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	53.20	27.47	39.42	26.41	157.15
3a	*Oe. pratincola* hyb. *immunis*	Resistant	100	81.92	29.15	52.77	102.30	243.25
4a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	55.72	29.14	16.34	40.89	165.05
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant	100	76.43	30.23	68.82	51.08	199.98
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible	100	48.97	26.96	50.59	16.40	101.33
13a	*Oe. mississippiensis* × *Oe. cinerescens*	do	100	58.35	21.45	----------	28.84	107.76

14	*Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid	do	100	59.21	20.43		25.29	95.99
24	*Oe. pratincola* mut. *nitidissima*	Resistant	100	74.90	26.72		104.98	291.17
25	do	Susceptible	100	60.02	26.59		72.90	224.92

TABLE 31.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, pentosan being taken as unity.*

Key No.	Name of strain.	Characteristic of strain.	Pentosan.	Total nitrogen.	Total sugar by acid hydrolysis.	Tannin.	Water-soluble acid.	Total ash.
1a	Resistant *Oe. pratincola* (a segregate from species hybrid)	Resistant	100	34.11	127.09	132.75	332.02	119.06
2a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	51.64	74.11	49.64	295.42	187.98
3a	*Oe. pratincola* hyb. *immunis*	Resistant	100	35.59	64.41	127.87	296.94	122.07
4a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	52.30	29.32	73.38	296.20	179.45
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant	100	39.56	90.05	66.83	262.41	130.84
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible	100	55.05	103.31	33.48	206.93	204.21
13a	*Oe. mississippiensis* × *Oe. cinerescens*	do	100	36.76		49.44	184.69	171.39
14	*Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid	do	100	34.50		42.70	162.11	168.87
24	*Oe. pratincola* mut. *nitidissima*	Resistant	100	35.68		140.15	388.72	133.50
25	do	Susceptible	100	44.32		121.46	374.75	166.62

TABLE 32.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, tannin being taken as unity.*

Key No.	Name of strain.	Characteristic of strain.	Tannin.	Pentosan.	Total nitrogen.	Total sugar by acid hydrolysis.	Water soluble acid.	Total ash.
1a	Resistant *Oe. pratincola* (a segregate from species hybrid)	Resistant	100	75.30	25.69	95.73	250.09	89.68
2a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	201.44	104.03	149.28	595.09	378.67
3a	*Oe. pratincola* hyb. *immunis*	Resistant	100	80.08	28.49	51.58	237.78	97.75
4a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	136.29	71.27	39.96	403.68	244.56
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant	100	149.63	59.19	134.74	392.64	195.77
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible	100	298.65	164.41	308.56	618.02	609.91
13a	*Oe. mississippiensis* × *Oe. cinerescens*	do	100	202.28	74.36		373.59	346.69
14	*Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid	do	100	234.18	80.79		379.65	395.47
24	*Oe. pratincola* mut. *nitidissima*	Resistant	100	71.35	25.46		277.37	95.25
25	do	Susceptible	100	82.33	36.49		308.54	137.18

TABLE 33.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, sugar being taken as unity.*

Key No.	Name of strain.	Characteristic of strain.	Total sugar by acid hydrolysis.	Pentosan.	Total nitrogen.	Tannin.	Water soluble acid.	Total ash.
1a	Resistant *Oe. pratincola* (a segregate from species hybrid)	Resistant	100	78.65	26.83	104.45	261.12	93.67
2a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	134.94	69.69	66.99	398.65	253.67
3a	*Oe. pratincola* hyb. *immunis*	Resistant	100	155.24	55.25	193.85	460.97	189.50
4a	Mildewed *Oe. pratincola* (a segregate from species hybrid)	Susceptible	100	341.00	178.34	250.23	1,010.12	611.97
8	*Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*	Resistant	100	111.05	43.93	74.21	291.39	145.29
9	*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*	Susceptible	100	96.79	53.28	32.41	200.28	197.66

In *Oenothera* it is well known that whole groups of morphological characters cohere strongly in inheritance. The explanation of the phenomenon is still a matter for investigation. Geo. H. Shull(44) thinks that he has reached a basis for an explanation in his hypothesis that the factorial differences between the various *Oenothera* strains in the congeries of elementary species and hybrids grouped about *Oe. biennis, Oe. pratincola, Oe. lamarckiana,* etc., are located in a single chromosome. Therefore, all factorial complexes are hereditary as a whole, except as crossing-over may occur. A more conservative hypothesis is that of Bartlett and of Cobb and Bartlett,(5, 10) according to whom the peculiarities of inheritance in *Oenothera* need not necessarily be viewed as dependent upon all the mutable factors being located in a single chromosome. On the contrary, several chromosomes may conceivably form a coherent group in the reduction division apart from a residue of other freely segregating chromosomes which are probably the bearers of typically Mendelian characteristics. The hypothesis thus briefly outlined is the one (called the α and β hypothesis) which has served for the formulation of the genetical data concerning the strains dealt with in this paper. It has been brought forward subsequently as something new by Lotsy,(29) who calls it the "Kernchimaera" hypothesis.

Without this slight digression the reader might misunderstand my conception of resistance to be that it is due to a complex of factors rather than a single factor. It is consistent with all that is known of the genetics of *Oenothera* for a factor complex to be inherited as a whole. Consequently, the breeding data are sharp and clear in a way that we seldom expect to find in other groups where the factors, which together contribute to immunity, are located in different chromosomes and, therefore, segregate freely from one another when hybridization takes place. In *Oenothera,* when a disease-resistant factor complex has once come about, the peculiarities of inheritance are such that it is not disintegrated even by hybridization; hence, the unusually sharp and simple relationships that have come to light with regard to inheritance. In plants with simpler, freely segregating Mendelian factors, one would expect very few cases in which clear-cut differences with regard to infection would prove to be due to a single Mendelian factor. The results with *Oenothera,* however, lead to the supposition that, if immunity is generally due to contributory and interacting factors, there is every reason why the plant breeder should work according to a consistent program

for the accumulation in a single strain of all factors contributing to disease resistance. In such work the chemical composition of the plant breeder's basic materials might afford him a criterion for guidance during the process of building up a resistant factor complex. The biochemical study of geneticists' materials certainly cannot fail eventually to find important applications.

It is instructive to compare the analytical data for reciprocal hybrids with those for mutations that differ visibly from each other only in resistance. The two members of the several pairs of reciprocal hybrids were usually quite distinct from each other morphologically as well as in resistance. They were selected as material for this study because they show great contrast in immunity, and the analytical data show correspondingly strong contrasts. In the case of the mutations, two types were chosen that were morphologically alike and differed only in resistance. Although called *Oe. pratincola* mut. *nitidissima*, neither was of direct descent from the original mutation, but they were the *nitidissima* types splitting out of crosses with the parent species, *Oe. pratincola*. Factorial recombination brought about forms differing in resistance. Comparison of the analyses shows variations in chemical composition quite parallel with those differentiating the more diverse reciprocal hybrids, but of smaller magnitude.

Another very interesting fact is brought out in the comparison of one member of a pair of reciprocal hybrids with a morphologically identical metaclinic hybrid in the reciprocal cross. To illustrate how these metaclinic plants arise, let us take a hypothetical case of a cross between species A and B, of which the matroclinic, true-breeding first generation hybrids are A′ and B′. Then

$$A \times B \rightarrow A'$$
$$B \times A \rightarrow B'$$

In rare instances, however, the progeny consisting of hybrid A′ may contain individuals of hybrid B′ and, conversely, the progeny consisting of hybrid B′ may contain a few individuals of A′. These plants which appear to be out of place are called metaclinic hybrids by De Vries.(17) In some instances, notwithstanding morphological identity with the majority of plants in the reciprocal cross, they will be different as to immunity. In such cases there are likewise chemical differences, of the same direction for the same constituents, as in the resistant and non-resistant types of *Oe. pratincola* mut. *nitidissima*.

In connection with the relative differences found in chemical composition between susceptible and resistant strains of *Oenothera,* it is of interest to give a brief account of the modes of entrance of the hyphae and haustoria of the Erysipheae into the cells of the host. Salmon(41) states that—

> the ordinary vegetative mycelium consists of very numerous, delicate, white or colorless septate hyphæ, frequently branched and more or less densely interwoven. In all genera, except Phyllactinia, the hyphæ of the vegetative mycelium produce haustoria at intervals which pierce the cuticle and swell out into a bladder-like form in the epidermal cells. These haustoria serve both to attach the fungus to its host and draw nourishment from it.

Smith(45) describes the haustorium of the Erysiphae, in the specific case of *Erysiphe communis* on *Geranium maculatum,* as

> a slender, proximal portion, the neck, penetrating the epidermal wall of the cell, within which it enlarges into a vesicular, distal portion with a thin wall. On the interior, the vesicle is filled with a delicate, spongy protoplasm differing in no visible particulars from the protoplasm in the mycelium.

In *Oenothera,* according to Klaphaak and Bartlett,(27) the mildew *Erysiphe polygoni* DC. grows very superficially and the haustoria of the fungus extend into the epidermal cells. It is, therefore, the epidermal tissue that should be examined if the most nearly perfect correlation of chemical composition with resistance is to be discovered. The composition of the whole leaf can be of significance only if there is a correlation between the composition of the epidermis and the underlying tissues. Such a correlation I have assumed, and the results are so consistent throughout that there is no reason to question the validity of the assumption. If we visualize the epidermal cells as comparatively rich in tannin, sugar, and water-soluble acids, we must bear in mind that either the actual synthesis of these substances occurs in the green cells or else the substances from which they are synthesized come from the green cells. The equilibrium between the chemical content of the epidermal cells and that of the underlying tissues is of such a nature that we must suppose transposition of material to take place whenever the concentration of water-soluble substances changes. The epidermis, unable to carry on fundamental chemical synthesis, must be in the most intimate equilibrium with other tissues, and we should expect differences in epidermal content to be correlated with differences in the leaf as a whole.

The rôle of tannin has been studied extensively, both from the point of view of the metabolism of the host and from that of its influence on parasites. Here we are not concerned with the question whether tannin is regarded as a by-product of metabolism or as a reserve material. The point of importance is whether or not it prevents infection by mildew. The results of the earlier investigations in this regard are conflicting. Cook and Taubenhaus(13) were able to demonstrate that tannin is toxic to the growth of certain fungi, the parasitic forms being more sensitive than the saprophytic. Wehmer(54) found that the resistence of oak wood to dry rot is correlated with the amount of tannin in the wood. On the other hand, Valleau(55) obtained negative results in his attempt to relate tannin to the differences in resistance of varieties of plums to brown rot. Moreover, Willaman and Sandstrom(51) did not find significant differences in the tannin concentration of normal plums and the ones rotted by *Sclerotinia cinerea*. The conflicting results may be due to differences in the localization of tannin in different parts of a plant or plant organ. No fact is more familiar to the geneticist than the existence of factors which determine the distribution in certain cells of pigment, tannin, enzymes, etc. Such factors give rise to the color patterns of flowers and animals. In *Oenothera*, the results show no evidence of disturbance due to such factors, but plums might present entirely different conditions. Tannin localized elsewhere than in the epidermis would have no influence on infection by the fungus which grows superficially in plant tissue. Analysis of a whole fruit might show a high tannin content but, nevertheless, the fruit might have no resistance. When the difference of the tannin content between resistant and susceptible plants fails to correlate with resistance, it may be that the tannin is differently located. The action of tannin as a protective agent is summarized by Thatcher(47) in the following words:

> Either the tannin actually serves as an antiseptic to prevent the growth of the parasitic fungus within the tissues of the host plant, or it assists in the development of a corky layer which "walls-off" the infected area and so prevents further spread of the disease through the tissue.

The fact that tannin coagulates albumin and other proteins, suggests that it may be a protoplasmic poison. When the fungi are grown in synthetic media containing varying amounts of tannin, the harmful effect of the tannin is manifested in the majority of cases. However, if tannin is injurious to fungus protoplasm, why is it not also harmful to the protoplasm of the

host plant? It is very likely that the protective effect of tannin in preventing the growth of parasitic fungi is conditioned by the other substances present in the cell sap. Thus De Dominicis (15) found that the coagulation of egg albumen by tannin can be prevented by the addition of acetic acid or tartaric acid, and that these two acids restore to its original condition an albumin which has been coagulated by tannin. Possibly in the tissues of a plant there are other substances besides the common acids which may serve to protect the protoplasm from the tannin of the same species. It is quite possible, however, that the foreign protoplasm of a parasitic fungus would be adversely affected by the same tannin. In this connection it is interesting to observe that an *Oenothera* that is high in tannin is likewise high in acid. Thus in the resistant strains we have a high tannin content, for which there is evidence of an immunizing function, coupled with a high acidity which is not inconceivably protective to the protoplasm. Thus an entire mechanism for resistance is present. Susceptible strains have a lesser development of the factorial complex for immunity, coupled with high water-soluble nitrogen which probably favors the nutrition of the mildew. One can hardly avoid the conclusion that striking differences in resistance are due to the interaction of various factors.

Most fungi grow best in slightly acid media but, when the concentration of acid is rather high, it inhibits growth. The cell-sap of the leaves of the resistant *Oenothera* is very high in acid which may influence the fungus adversely, quite aside from its possible protective influence in preventing coagulation of the proteins of the host protoplasm. The relation of acid content of the plant to disease resistance has been pointed out by many investigators. Comes(11) found that Rietti wheat, a very resistant variety, has a higher percentage of acid than other, susceptible varieties. Moreover, he also observed that, as the rust resistance of wheats increases with the altitude at which they are grown, their acid content is also correspondingly increased. Averna-Sacca(3) found that American grape and other plants which are highly resistant to the attacks of *Oidium peronospora* and gall mites are characterized by having a very high acid content. Brown(6) reported that the enzyme activity of an extract from *Botrytis cinerea* is inhibited by the addition of a fair amount of acid. The high concentration of acid in the leaves of the resistant *Oenothera* may be correlated with its resistance.

Turning now to the susceptible plants, we find that their nitrogen and ash content is high. Analyses of the constituents

of total nitrogen and ash reveals further that, in the case of nitrogen, the form that predominates is the water-soluble nitrogen which, in turn, has a high concentration of amino acids and of nitrogenous compounds of nonbasic character. The ash contains a great deal of calcium and sulphur.

Zellner(57) found that Ascomycetes contain much protein but that the amount of tannin, if ever present, is extremely low. According to Pfeffer,(38) most fungi that are able to develop, in media containing nitrogen in the form of ammonium nitrate, grow better when supplied with peptone, amides, and other nitrogenous organic compounds. That the mildew on *Oenothera* probably derives its supply of nitrogen mainly from the water-soluble nitrogenous compounds in the leaves is sustained by the following experimental observation. When susceptible and resistant plants are examined at the period of their highest stage of growth, during which time the mildew on their leaves is also very active, the difference of nitrogen content between resistant and susceptible strains lies in the water-soluble nitrogen. In the same plants, examined at the time when they approach senility, the relative difference in nitrogen still persists but the consistent difference is found in the protein nitrogen. There are two possible explanations to account for this. One is that, during infection, the mildew is continually using some of the simple nitrogenous compounds, perhaps some of the amino acids, leaving other amino acids in excess, which are not immediately utilized for the synthesis of proteins. The equilibrium in the host may be looked upon as disturbed by the withdrawal of protein-forming substances in proportions different from those in which they are utilized in protein synthesis. In the senescent leaves, on the contrary, the mildew is no longer growing and the normal metabolism of the plants is not disturbed. The other explanation is that the formation of simple nitrogenous compounds takes place so rapidly during the height of the growth period that protein synthesis lags somewhat behind. In other words, material for protein synthesis is produced much faster than it can be utilized. The second explanation seems to be the more plausible one. Certainly the high nitrogen content of the susceptible plants would seem to have something to do with their infection by the mildew. Otherwise, they would not be so much more susceptible at one time than at another.

Calcium, to which the high ash content of susceptible *Oenothera* is largely due, is present in the living plant in the form of raphides of calcium oxalate. Its presence may indirectly benefit

the mildew by neutralizing some of the acids in the cell sap. However, complicated questions of plant physiology are here concerned, and the suggestion would hardly be made if it were not for the definite correlation of high ash content with low acidity. Bartlett informs me that the most heavily mildewed *Oenothera* plants he has ever seen were on shingle of a river bank at the base of limestone cliffs. The fact that the total ash is considerably higher in the susceptible plants than in the resistant plants indicates that possibly the rate of absorption of the inorganic elements differs considerably. Since the inorganic salts absorbed from the soil together with the carbon dioxide taken in from the atmosphere constitute the raw materials for the synthesis of food in the plant, it seems very suggestive that the causes for the differences in the amount of elaborated constituents can be traced back to differences in salt absorption.

A chemical basis for true immunity does not necessarily imply the presence of some toxic substances in the resistant plants and the complete absence of such substances in the susceptible plants; conversely, it does not imply the presence of some chemotactic substance in the susceptible plants that does not exist in the resistant ones. Differences of the concentration of these substances in the sap, as well as their localization in the tissues of the plant, are two of the main factors to be considered. A resistant plant and a susceptible one may both contain the same toxic substances or chemotactic substances; but, if the amount of such substances present in one or the other is small, or if they are differently located, then they may exercise no harmful or beneficial influence on the fungus. The effect of the concentration of the substances in the cell sap is well shown in the experimental results obtained with the susceptible and the resistant strains of *Oenothera,* while the effect due to difference of location of the substances in the tissues of the plant is purely an expression of opinion arising from the fact that not all other investigators have been able to establish definite correlations between composition (especially with regard to tannin) and resistance to infection.

SUMMARY AND CONCLUSIONS

1. Chemical analyses of several resistant and susceptible hybrids and elementary species of *Oenothera* were made in order to discover whether or not there were definite correlations between chemical composition and reaction to mildew. The chemical analyses were also extended to other plants which were

found to have strains resistant and susceptible to other forms of powdery mildew.

2. The resistant strains are characterized by being higher in tannin and water-soluble acid. In the majority of cases, they are also higher in crude fiber than the susceptible ones.

3. The susceptible strains are comparatively high in total nitrogen and total ash.

4. The forms of nitrogen that are high in the susceptible strains during infection are the amino acids and nitrogenous compounds of nonbasic character. When the plants are approaching senility, the leaves of the susceptible plants are extremely rich in protein nitrogen.

5. The ash of the susceptible plants is exceedingly high in calcium and sulphur, as compared with that of the resistant plants.

6. All the strains examined, comprising wild elementary species, reciprocal hybrids, and mutations, show the same correlation of chemical composition with disease resistance.

7. The differences in chemical composition are found in both resistant and susceptible strains, before infection and after, and during the period when the leaves of the plants show chlorophyll degradation, indicating that the characteristic features of the resistant and the susceptible strains are constitutional.

8. The chemical analyses made of the susceptible and the resistant plants of *Syringa vulgaris*, *Desmodium canadensis*, *Helianthus giganteus*, and *Solidago canadensis* confirm the findings in regard to *Oenothera*. An exception was found in the total ash and water-soluble acid contents of *Helianthus giganteus*, but the plants were wild and may not have been genetically as close as appearance indicated.

9. Resistance in plants is doubtless due to a complex of interacting factors. Factors that increase tannin and acid and that decrease water-soluble nitrogenous compounds tend to build up immunity.

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APPENDIX

RECORD OF THE PARENTAGE OF ALL STRAINS OF OENOTHERA USED IN THE EXPERIMENTS

[C always indicates the type strain of *Oenothera pratincola* Bartlett known as "Lexington C." Similarly D indicates *Oe. numismatica* Bartlett, known as "Lexington D." See Bartlett (4) 1915.]

0847[1] = C–52–6–25–1–41 *typica*.....
× 7–19
C–22–15 × C–22–10 } 1–25–34 *simulans*.....
× = resistant *Oe. pratincola* (a segregate from species hybrid).
CD–9–49–6 *typica*..... × CD–9–9–46 *simulans* } 2–33–1.....
(= reciprocal of 0822.)

1827 = 0847–4.

2960 = 0847–4–10.

0822 = CD–9–49–6 *typica*..... × CD–9–9–46 *simulans*.. } 2–33–1.....
× = mildewed *Oe. pratincola* (a segregate from species hybrid).
C–52–6–25–1–41 *typica*.....
× 7–19
C–22–15 × C–22–10 } 1–25–34 *simulans*.....
(= reciprocal of 0847.)

1824 = 0822–26.

2959 = 0822–26–34.

0846 = C–52–6–25–1–41 *typica*.....
× 7–21
C–22–15 × C–22–10 } 1–25–34 *simulans*.....
× = *Oe. pratincola* hyb. *immunis*.
CD–9–49–6 *typica*..... × CD–9–9–46 *simulans*.. } 2–33–22.....
(= reciprocal of 0823.)

[1] These numbers represent "culture number" of the strain; that is, the number under which a progeny was grown in the garden.

1826 = 0846–2.
2956 = 0846–2–31.
0823 = CD–9–49–6 *typica* × CD–9–9–46 *simulans* 2–33–22 × C–52–6–25–1–41 *typica* × C–22–15 × C–22–10 1–25–34 *simulans* 7–21 = mildewed *Oe. pratincola* (a segregate from species hybrid).
(= reciprocal of 0846.)
1825 = 0823–5.
2954 = 0823–5–61.
0830 = Cartersville 13d–1–22–24–5–6 × CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* 2–33–23 = *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*.
(= reciprocal of 0830.)
1804 = 0830–7.
0820 = CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* 2–33–23 × Cartersville 13d–1–22–24–5–6 = *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*.
(= reciprocal of 0830.)
1805 = 0820–36.
0829 = Cartersville 13d–1–22–24–5–7 × CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* 2–33–23 = *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*.
(Similar to 0830.)
1818 = *cinerescens* (2) 9–3_{16}–5–20–1 × CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* 2–33–17 60 = *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. (Matroclinic in morphology.)
(= F_1 of reciprocal cross of parents of 1819.)
2966 = 1818–75.
1819 = CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* 2–33–17 × *cinerescens* (2) 9–3_{16}–5–20–1 4 = *Oe. pratincola* mut. *immunis* × *Oe. cinerescens*. (Matroclinic in morphology.)
(= F_1 of reciprocal cross of parents of 1818.)

2963 = 1819–18.

0838 = "*biennis Chicago*" 2–20–3–1 × CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* (2–33–19) = *Oe.* "*biennis Chicago*" × *Oe. pratincola* hyb. *immunis.*

(= reciprocal of 0821.)

0821 = CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* (2–33–19) × "*biennis Chicago*" 2–20–3–1 = *Oe. pratincola* hyb. *immunis* × *Oe.* "*biennis Chicago.*"

(= reciprocal of 0838.)

0814 = *cinerescens* (2)–9–3_{16}–5–20–3 × Cartersville 13d–1–22–24–5–5 = *Oe. cinerescens* × *Oe. mississippiensis.*

(= reciprocal of 0827.)

1802 = 0814–64 = *Oe. cinerescens* × *Oe. mississippiensis* (metaclinic).

2958 = 0814–64–27.

0827 = Cartersville 13d–1–22–24–5–5 × *cinerescens* (2)–9–3_{16}–5–20–3 = *Oe. mississippiensis* × *Oe. cinerescens.*

(= reciprocal of 0814.)

1800 = 0827–12.

2957 = 0827–12–12.

0299 = C–52–6–31–73–19–6 *typica* × (C–52–6–31–72–3 *typica* × *reynoldsii typica debilis* 56–21) (9) (2) = *Oe. pratincola* × *Oe. reynoldsii* (*Oe. pratincola* hyb. *amycosa.*)

(Similar in genetic constitution to reciprocal of 0177.)

0177 = *reynoldsii typica debilis* 56–21 × C–52–6–31–73–3 (6–4) = *Oe. reynoldsii* × *Oe. pratincola.*

0842 = "*biennis Chicago*" 2–20–3–2 × (C–52–6–25–1–41 *typica* × (C–22–50 × C–22–10) 1–25–34 *simulans*) (7–22) = *Oe.* "*biennis Chicago*" × *Oe. pratincola* hyb. *rubricalyx.*

(= reciprocal of 0849.)

1816 = 0842–4.

2962 = 0842–4–10.

0849 = C–52–6–25–1–41 *typica*
× 7–22 = *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."*
C–22–15 × C–22–10 } 1–25–34 *simulans*
× *"biennis Chicago"* 2–2–3–2
(= reciprocal of 0842.)

1817 = 0849–8.

2961 = 0849–8–12.

0831 = Cartersville 13[d]–1–22–24–5–3
× = *Oe. mississippiensis* × *Oe. "biennis Chicago."*
"biennis Chicago" 2–20–3–1
(= reciprocal of 0839.)

1806 = 0831–10.

0841 = *"biennis Chicago"* 2–20–3–1
× = *Oe. "biennis Chicago"* × *Oe. mississippiensis.*
Cartersville 13[d]–1–22–24–5–3
(= reciprocal of 0831.)

0839 = *"biennis Chicago"* 2–2–3–2
× = *Oe. "biennis Chicago"* × *Oe. mississippiensis.*
Cartersville 13[d]–1–22–24–5–8
(= similar to 0841.)

1807 = 0839–2.

0833 = Cartersville 13[d] 1–22–24–5–6
× = *Oe. mississippiensis* × *Oe. pratincola* hyb. *rubricalyx.*
CD–52–6–25–1–41 *typica* × 7–22
C–22–15 × C–22–10 } 1–25–34 *simulans*
(= reciprocal of 0848.)

1810 = 0833–6.

0848 = C–52–6–25–1–41 *typica*
× 7–22 = *Oe. pratincola* hyb. *rubricalyx* × *Oe. mississippiensis.*
C–22–15 × C–22–10 } 1–25–32 *simulans*
× Cartersville 13[d]–1–22–24–5–6
(= reciprocal of 0833.)

1811 = 0848–60.

1346 = E–5–208–1–182 *nitidissima*
× 7–1–10 = *Oe. pratincola* mut. *nitidissima.* (Resistant.)
E–43–74–21 *typica*

1348 = same as 1346.

1347 = E–5–208–1–182 *nitidissima*
× 7–1–11 = *Oe. pratincola* mut. *nitidissima.* (Mildewed.)
E–43–74–21 *typica*

1349 = same as 1347.

199 = D–1–11–24–28–45–34–9–25 ..... = *Oe. numismatica.*

1191 = CD–9–9–42–31–572–1–11 ..... = *Oe. pratincola* mut. *simulans.*

1192 = (C–22–15 × C–22–10) 1–24–2–29–9–4 ..... = *Oe. pratincola* mut. *simulans rubricalyx.*

1829 = (CD–9–49–6 *typica* ..... × CD–9–9–46 *simulans* .....) 2–33–1–8 ..... = *Oe. pratincola* hyb. *immunis.*

2953 = 1829–36.

1231 = (*reynoldsii typica debilis* 56–21 × C–52–6–31–73–3 *typica* .....) 6–7–11 ..... = *Oe. reynoldsii* × *Oe. pratincola.*

167 = C–52–6–25–1–43–2–22–4 *typica* ..... = *Oe. pratincola* (susceptible.)

1828 = Cartersville 13[d]–1–22–24–5–9–7 ..... = *Oe. mississippiensis.*

1195 = (C–52–6–25–1–41 *typica* ..... × (C–22–15 × C–22–10) 1–25–34 *simulans rubricalyx* .....) 1–7–3 = *Oe. pratincola* hyb. *rubricalyx.*

1234 = 89–85–40–46–44–22–11 *typica reynoldsii* ..... = *Oe. reynoldsii.*

2950 = "*biennis Chicago*" 2–20–3–1–1–2 ..... = *Oe.* "*biennis Chicago.*"

2952 = (C–52–6–25–1–4 *typica* ..... × (C–22–15 × C–22–10) 1–25–34 *simulans* ..) 7–19–21–27 = *Oe. pratincola* hyb. *rubricalyx.*

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